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IMPROVED RATE OF DRY MATTER DISAPPEARANCE (SLOW DMD) IN SASKATCHEWAN BARLEY FOR BEEF AND DAIRY CATTLE

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Improved Rate of Dry Matter Disappearance (Slow DMD) in Saskatchewan Barley for Beef and Dairy Cattle

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Abstract

Barley grain is an important source of energy and protein for ruminant animals. However, feeding must be carefully managed to avoid acidosis, liver abscesses and bloat caused by the rapid breakdown of barley starch in the rumen. The development of slower degrading barley could aid in alleviating health problems associated with consumption of barley grain. Selection for hard endosperm may result in slower starch degradation and improved feed quality. This study examined the effect of grain hardness, variety and environment on dry matter disappearance rate (DMDR); attempted to identify accurate and efficient grain hardness selection tools; studied environmental effects, inheritance and heritability of grain hardness, and undertook development of locally adapted barley germplasm with slow DMDR."

To study grain hardness, genetic and environmental effects on DMDR, two genotypes grown at multiple locations in 2004 were analyzed for SKCS hardness, by scanning electron microscopy and for *in situ* dry matter disappearance rate. Genotype by environment interaction influenced DMDR, while neither SKCS hardness nor SEM analysis accurately differentiated DMDR between genotypes.

Eight genotypes were grown at multiple locations during 2003 and 2004 to study grain hardness measurement methodology and genetic and environmental effects on hardness. Genotypes were analyzed for SKCS hardness, milling energy, light reflectance, feed particle size, protein and beta-glucan. Hardness measurements, including SKCS hardness, Milling Energy, and light reflectance ranked genotypes similarly across environments. Feed particle size was correlated with milling energy but not other hardness measurements. Hardness measurements appeared influenced by protein and beta-glucan.

To examine the inheritance and heritability of barley grain hardness, 245 double haploid (DH) genotypes, grown in 2003 and 2004, were analyzed for SKCS hardness, milling energy, protein and beta-glucan, with 100 evaluated for light reflectance. The population exhibited normal distributions for SKCS hardness, milling energy, protein and beta-glucan, suggesting quantitative inheritance for these traits with no apparent epistastic gene interaction. Transgressive segregation was apparent beyond both parents for SKCS hardness, milling energy and protein. Heritability was 0.75 for SKCS hardness and 0.41 for protein. Light reflectance was not normally distributed, suggesting complementary gene interaction. Broad-sense heritability was 0.53.

Barley grain hardness is heritable and could be used as an efficient tool in making selections in a breeding program. However, more research is required to elucidate the relationship between DMDR and grain hardness.

Hundreds of putative slow DMDR selections were developed for use in the CDC breeding program. The most advanced were based on the reported slow DMDR variety Valier from Montana State University. However the results of this project indicate its slow DMDR character is not consistently expressed retrospectively making it a poor

choice as a slow DMDR donor parent. As a result the majority of Valier derivatives have been dropped from the germplasm development program and will not be utilized in the CDC breeding program. Hulled two-row derivatives from the very hard grain, hulless, six-row putative slow DMDR parents from the Barley World Collection, WC795 and WC1064, have reached advanced germplasm development and evaluation stages of the program but have yet to be specifically evaluated for DMDR.

Executive Summary

The initial objectives of this project were to develop rapid, small sample screening technology to select for slow rate of ruminal dry matter disappearance (slow DMDR) in barley and to develop locally adapted barely germplasm with the slow DMDR trait.

While the project was plagued with difficulty including the early departure of an MSc candidate and damaging weather issues in 2002 and 2004, all of which significantly delayed the project, the greatest set back was determined by the research project itself. That was the discovery that the variety Valier, reported to be a slow DMDR type, in fact shows inconsistent performance due to significant environmental effects on the slow DMDR trait. This resulted in much of the material being developed and studied under the project also being inconsistent for DMDR meaning our original objective regarding method development to screen for and evaluate slow rate DMD could not be achieved.

Despite this set back we did learn several things from the project and its off-shoots especially in terms of evaluation of barley grain quality as measured by SKCS grain hardness, Milling Energy, Scanning Electron Microscopy and light reflectance and were able to develop germplasm with potential slow DMDR.

While, due to field sprouting essentially no slow DMD analysis could be conducted from 2002 experiments, sufficient seed was salvaged and by growing unscheduled catch-up 2002/03 New Zealand winter increases, sufficient samples were available for analysis from 2003 and 2004 experiments. Negative weather effects of the 2004 harvest season led to severe harvest delays and subsequent late data collection. This led to the extension (without additional funding) of the project through 2006. While 2005 and 2006 saw the grow out of a number of sets of materials associated with the germplasm development sub-project, no further samples were required for completion of the slow DMD project first objective.

In terms of methodology development difficulties encountered with the GSPA (gross particle size analysis) system being developed for base phenotyping led to a significant change in workplan in which several in vitro digestibility studies were completed in collaboration with Dr. John McKinnon, Animal & Poultry Science, U of S, to re-evaluate the slow DMDR of Valier versus normal barley genotypes. The overall result of these studies was to conclude that an in vitro study was justified. That in-vitro experiment was conducted in collaboration with Drs. McKinnon and Pei-chung Yu in 2005 when fistulated steers were available in the Department of Animal & Poultry

Science. Unfortunately the results confirmed our suspicions that our base genetic resource for the slow DMDR trait, the variety Valier, was in fact highly inconsistent in expression of the trait depending on growing environment. This led to overall difficulty in achieving our initial objectives.

Collaborative ME (milling energy) evaluations of selected project materials with colleagues at the SCRI (Scottish Crop Research Institute) in the UK delivered interesting results indicating that ME measures may be associated with DMDR and with our local SKCS (Single Kernel Characterization System) grain hardness measures. 2004 studies conducted on 2003 grown samples were repeated in 2005 on 2004 grown samples and verified the value of the ME measure and its relation to SKCS hardness. 2005 and 2006 saw further evaluation of short cut screening methods including SKCS, rate of water uptake, light reflectance, evaluation of energy required to roll various variable grain samples from our trials as well as additional scanning electron microscopy to rationalize the results of our in-vitro study of Valier and TR253.

As previously reported, after the sudden departure of MSc candidate S. Alwala in 2003, Ms. Giselle Camm decided to pursue her MSc on a part time basis and took up a significant portion of the project for her MSc studies.

Ms. Camm's objectives, which melded well with the first ADF project objective, were:
(1) To identify a simple, effective method(s) to screen breeding program germplasm for slow rate of DMD based on gross particle size, acid extract viscosity, Rapid Visco Analysis (RVA), the Perten Single Kernel Characterization System (SKCS), rate of water uptake or other methodology which can distinguish grain texture differences
(2) To study the inheritance of the slow DMD trait in a doubled haploid barley populations with Valier as a slow DMD parent

Ms. Camm's MSc thesis project and thesis is at the penultimate thesis draft stage and will be completed in late 2007 or early 2008. In addition to revealing that Valier's DMDR was not consistently expressed across growing environments both by conducting animal experiments and using Scanning Electron Microscopy, her results indicate that SKCS grain Hardness is a reasonably heritable trait (Heritability = 0.75) meaning it can be effectively used to select for specific genetic levels of grain hardness in barley. SKCS Hardness was not as strongly associated with DMDR as anticipated, however it still is a good candidate for such quality selection at the breeding level. Her work also indicated that Milling Energy, which showed more association with grain particle size than other measures evaluated, may be a trait which could be used to select for differential DMDR. While not as simple and convenient from a screening methodology viewpoint it does represent a possible indirect DMDR screening method.

With regard to the second objective, putative slow DMDR materials derived from slow DMDR parents Valier, WC 795 and WC1064 were evaluated at all stages of the CDC barley breeding program through 2006 with the most advanced derivatives in 2004 thru 2006 yield trials at as many as six western Canadian locations. Since Valier retrospectively turned out to be a poor choice as a slow DMDR donor parent few

selections from crosses with it have been continued in the program. However, based on information garnered throughout this project several selections from crosses involving the putative slow DMDR parents WC795 and WC1064 which demonstrate unique grain quality features have been advanced in the germplasm development program and in the CDC feed barley breeding program. Since WC795 and WC1064 are six-rowed and hulless and our overall breeding objective is to create slow DMDR hulled two-row genotypes the breeding process is more difficult and time consuming.

Technical Report

Screening technology

Project Objective #1 – "To develop rapid, small sample, screening technology to select for slow rate of ruminal dry matter disappearance (slow DMD) in barley."

As previously reported, after the sudden departure of MSc candidate S. Alwala, Ms. Giselle Camm, a technical assistant with our program since 1996, decided to pursue her MSc on a part time basis and took up a significant portion of the project for her MSc studies.

Ms. Camm's objectives were similar to Mr. Alwala's and melded well with the overall ADF project objectives, in particular Project Objective # 1.

(1) To identify a simple, effective method(s) to screen breeding program germplasm for slow rate of DMD based on gross particle size, acid extract viscosity, Rapid Visco Analysis (RVA), the Perten Single Kernel Characterization System (SKCS), rate of water uptake or other methodology which can distinguish grain texture differences.
(2) To study the inheritance of the slow DMD trait in a doubled haploid barley population with Valier as a slow DMD parent.

Efforts to evaluate dry rolling followed by RoTap sieve separation, to differentiate between the two starch protein matrix types (we have termed this base screening technique Gross Particle Size Analysis or GPSA), continued to struggle early in 2004. Difficulty was experienced in obtaining repeatable results from samples of Valier, CDC Helgason and TR253 (the parents of the populations being evaluated) from our 2003 experiments (2003 BarVYT) grown at six sites in MB, SK and AB. While we had planned to use this methodology to "phenotype" all materials in the project for the slow DMD vs normal trait it was necessary to alter our workplan to ensure that the problems were not with variability among samples rather than the methodology being developed.

This alteration led to a significant collaboration with Dr. John McKinnon and his group in Animal & Poultry Science using in vitro digestion testing to evaluate several sample sets of Valier, TR253 and CDC Helgason for DMD rate to ensure that the reported slow rate DMD trait of Valier was consistent across sites. Three experiments were conducted.

During March 2004 a preliminary in vitro digestion trial was conducted using samples of Valier, TR253 and CDC Helgason from 2003 Saskatoon and Goodale trials. Each sample was tested both as quartered and ground grain using two replicates. Rate of dry matter disappearance was determined after 0, 3, 6 and 12 hours of in-vitro digestion. Unfortunately experimental error was high and no conclusions could be drawn. During June a second experiment was conducted using the same entries but in this case only quartered grains were evaluated and the experiment had three replicates. In this study error levels were acceptable but results were inconsistent across samples and locations. During August a third similar in vitro experiment was conducted except that in this

instance all samples were ground, but once again results were inconclusive and variable across genotypes and growing sites.

The overall conclusion from this frustrating set of time consuming and expensive additional testing was that we needed to conduct an in-vitro rate of DMD disappearance trial. That resource intensive experiment was carried out during February and March 2005. This experiment evaluated only Valier versus TR253 but from 4 growing locations with all samples rolled rather than quartered or ground. DMD rate was determined after 0, 2, 4, 8, 12 and 24 hours. While it had been hoped this trial would confirm the slower DMD rate of Valier previously demonstrated in trials at Montana State University the results were at best mixed in that depending on growing environment Valier only sometimes demonstrated the slow DMD characteristic. As described below, the disappointing results of this in-vivo study indicated that Valier was actually an unfortunately poor choice of germplasm from which to attempt to capture the slow DMD trait. As a result the direction of our studies began to focus more on the study of grain hardness and methods to evaluate same. Additional effort was also expended to collect more scanning electron microscopy data to attempt to visualize and rationalize the inconsistent results for Valier in the in-vivo study.

SKCS hardness and gross particle size

Results

Table 1 shows no differences between Valier and TR253 for protein or feed particle size while differences were detected for SKCS hardness ($P<0.0006$), with Valier having harder grain.

Table 1 Mean SKCS hardness, protein and feed particle size of Valier and TR253, grown at four sites in 2004.

	Genotype			
Trait	Valier	TR253	SE	Pr>t
SKCS Hardness	53.3	49.1	0.28	0.0006
Protein	11.3	10.7	0.27	0.12
% Particles > sieve size				
3.35 mm	26.6	22.0	3.91	0.32
2.36 mm	48.8	50.1	1.74	0.52
2.00 mm	13.3	14.9	1.74	0.43
1.40 mm	6.59	7.93	0.71	0.16
1.00 mm	2.06	2.34	0.12	0.11
<1.00 mm	2.64	2.78	0.17	0.46
Mean Particle Size (μm)	3033	2831	149	0.27

A summary for Valier and TR253 grown at Brandon, Saskatoon (KCRF and SF) and Wakaw is given in Table 2. SKCS hardness was highest at Saskatoon (KCRF) (57.0)

site, followed by Wakaw, Brandon and Saskatoon (SF) (51.3, 48.5, 48.1, respectively). Similarly, the Saskatoon (KCRF) site had the largest percentage of particles >3.35-mm and mean particle size after rolling, with Wakaw, Brandon and Saskatoon (SF) following (Table 3.3). Brandon had the largest percentage of particles <1.00-mm compared to Saskatoon (SF), Wakaw and Saskatoon (KCRF).

Table 2. Brandon, Saskatoon (KCRF and SF) and Wakaw site summary for Valier and TR253 protein and feed and mean particle sizes, grown in 2004.

Site	Genotype	SKCS Hard	% Protein	% Particles > sieve size						Mean particle size (μm)
				3.35 mm	2.36 mm	2.00 mm	1.40 mm	1.00 mm	<1.00 mm	
Brandon	Valier	**50.9**	10.5	20.0	52.3	15.4	7.00	2.23	3.17	2791
	TR253	46.1	9.8	21.7	53.7	12.6	6.76	2.24	3.07	2874
	Mean	48.5	10.2	20.9	53.0	14.0	6.88	2.24	3.12	2833
Saskatoon (KCRF)	Valier	59.1	11.5	39.4	43.4	9.6	4.17	1.39	2.04	3556
	TR253	54.9	11.0	30.1	47.1	12.7	6.13	1.79	2.18	3116
	Mean	57.0	11.3	34.8	45.3	11.2	5.15	1.59	2.11	3336
Saskatoon (SF)	Valier	50.3	11.7	14.9	54.2	16.1	9.07	2.59	3.08	2618
	TR253	45.8	10.5	17.3	50.5	16.8	9.71	2.74	2.99	2643
	Mean	48.1	11.1	16.1	52.4	16.5	9.39	2.67	3.04	2631
Wakaw	Valier	53.0	11.4	32.1	45.3	12.1	6.12	2.04	2.27	3168
	TR253	49.6	11.5	19.0	49.0	17.5	9.11	2.58	2.88	2689
	Mean	51.3	11.5	25.6	47.2	14.8	7.62	2.31	2.58	2929

Scanning Electron Microscopy (SEM)

(SEM) of endosperm ultrastructure revealed differences between Valier and TR253 (Figure 1), but these were not consistent across sites. In prismatic endosperm cells from Brandon (Figure 1A-1B), Valier A and B-type starch granules (1A) were extensively enveloped in a sheet of starch-associated protein, resulting in an almost continuous starch-protein matrix. Individual endosperm cells were indiscernible. Comparatively, TR253 A and B-type granules (1B) were readily exposed, with minimal associated protein. Individual cells were easily distinguishable while, in other middle endosperm areas of TR253, pockets of cells with starch associated protein were identified (not

shown). These areas had less starch-associated protein than the corresponding Valier matrix as B-type granules were dislodged from the TR253 matrix and not from Valier.

Conversely, in samples from Saskatoon (KCRF) (Figure 1C-1D), TR253 (1D) starch was extensively coated with protein, masking A and B-type granules and individual endosperm cells. Valier's endosperm cells were readily distinguishable and appeared intact, with minimal disruption of A and B-type granules (1C). Protein coating was not evident.

In samples from Saskatoon (SF), minimal differences in the starch-protein matrix were noted between Valier (1E) and TR253 (3F) (Figure 1E-1F). Both had limited starch-protein association with B-type granules dislodged from the cells. Individual cells were readily apparent.

In samples from Wakaw (Figure 1G-1H), Valier endosperm cells (1G) appeared intact with minimal granule disruption. Granules were extensively enveloped in protein, resulting in an almost continuous starch-protein matrix. Comparatively, TR253 (1H) had more cellular disruption as B granules were dislodged from the starch-protein matrix. Patches of cells with starch-associated protein were scattered throughout the middle endosperm (not shown). TR253 (1H) had more cellular disruption as B granules were dislodged from the starch-protein matrix. Patches of cells with starch-associated protein were scattered throughout the middle endosperm (not shown).

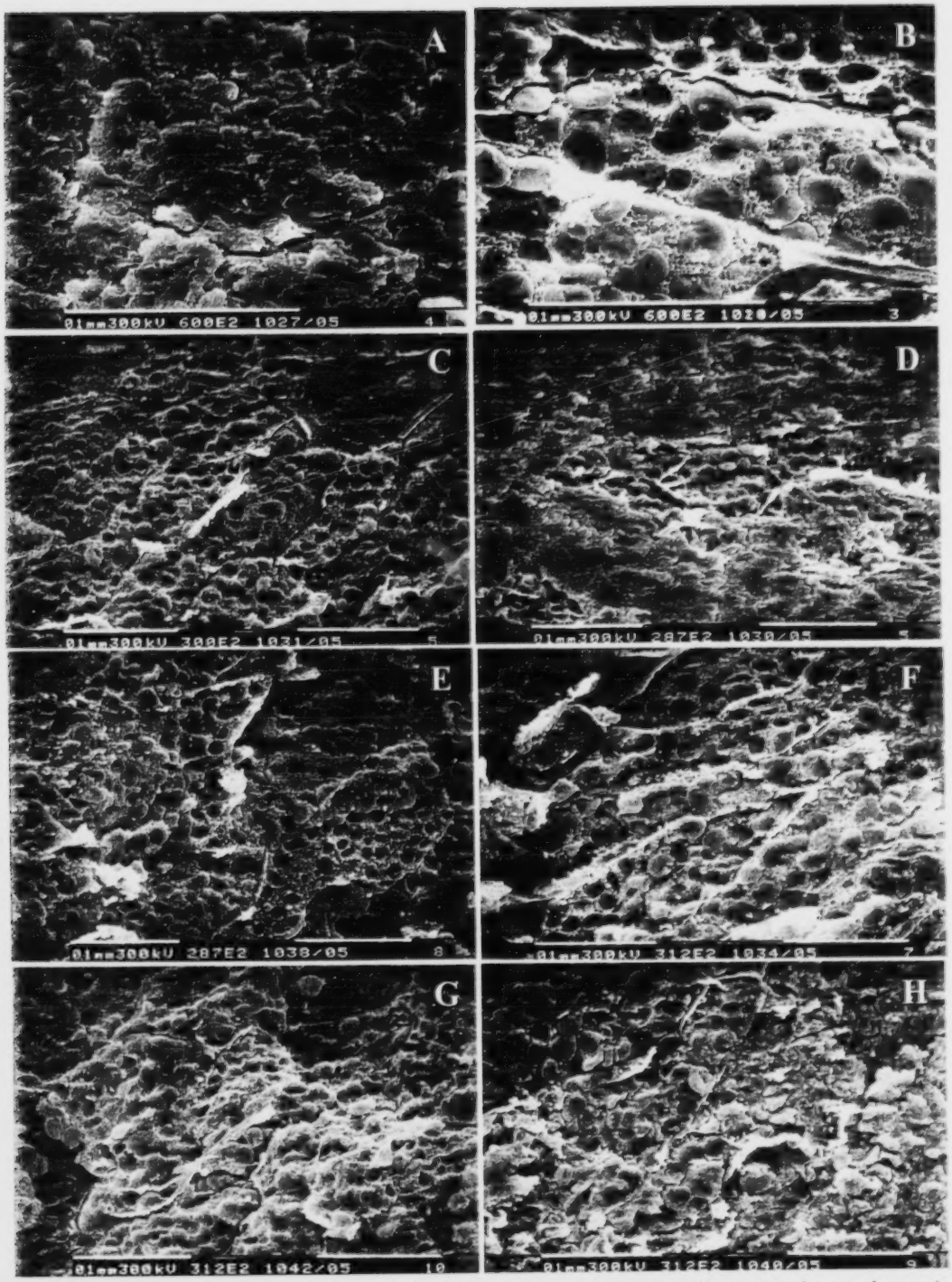

Figure 1. SEM micrographs of Valier and TR253 prismatic endosperm illustrating the starch-protein matrix of the genotypes grown at four sites in 2004: Valier (A) and TR253 (B) at Brandon; Valier (C) and TR253 (D) at Saskatoon (KCRF); Valier (E) and TR253 (F) at Saskatoon (SF); Valier (G) and TR253 (F) at Wakaw.

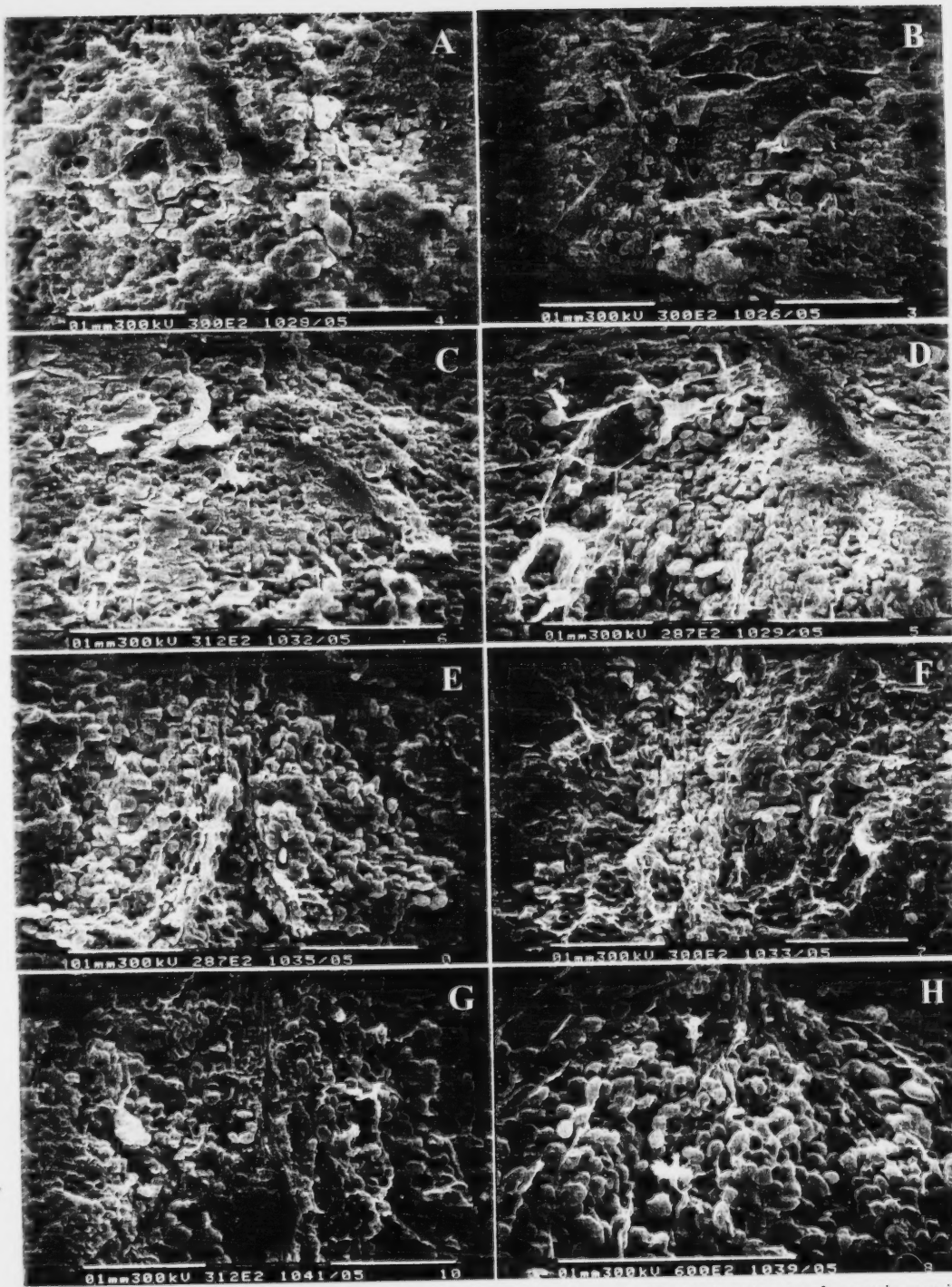

Figure 2. SEM micrographs of Valier and TR253 central endosperm illustrating the starch-protein matrix of the genotypes grown at four sites in 2004: Valier (A) and TR253 (B) at Brandon; Valier (C) and TR253 (D) at Saskatoon (KCRF); Valier (E) and TR253 (F) at Saskatoon (SF); Valier (G) and TR253 (F) at Wakaw.

In central endosperm areas, SEM revealed further differences between Valier and TR253 (Figure 2). Again, differences were not consistent across growing location. From Brandon, central endosperm areas (Figure 2A-2B) of Valier (2A) had large amounts of starch-associated protein and a compact structure, whereas TR253 (2B) endosperm was more open with loose A-type granules. Protein coating was evident in some areas, but less than Valier. Similarly for Saskatoon (KCRF) (Figure 2C-2D) and Wakaw samples (Figure 2G-2H), Valier (2C and 2G) A-type granules were noticeably coated with protein and densely packed into the cells, in contrast with TR253 (2D and 2H), which had a more open structure and loose granules. However, from Saskatoon (SF) (Figure 2E-2F), TR253 (2F) samples appeared compact with more protein-associated starch granules compared to Valier (2E), which had an open structure with limited protein sheeting. Few B-type granules were present in any central region.

In situ Rumen Degradation Results

Factorial analysis of variance of genotype x site revealed no significant differences between genotypes for Kd (degradation rate), A (rapidly soluble fraction), EDDM (effective degradation of dry matter) or RUDM (rumen undegradable dry matter) ($P>0.21$) (Table 5). However, significant differences were detected between genotypes for B (potentially degradable fraction) and C (undegradable fraction) fractions ($P<0.06$). Valier had a greater potentially degradable fraction (B) than TR253 (62.1 versus 56.5%, respectively) while TR253 had a greater undegradable fraction (C) than Valier (34.6% versus 29.4%, respectively).

Table 5. Valier and TR253 in situ dry matter disappearance characteristics, from four sites in 2004.

Genotype	Kd (%/hr)	A fraction (% DM)	B fraction (% DM)		C fraction (% DM)		EDDM (%)	RUDM (%)
Valier	11.4	8.5	62.1	b	29.4	a	48.8	51.2
TR253	14.3	8.9	56.5	a	34.6	b	47.9	52.1
Mean	12.9	8.7	59.5		31.8		48.4	51.6
SE	0.93	0.19	1.66		1.64		0.56	0.56

Significance level P<0.10.

Location differences were detected for EDDM and RUDM ($P<0.06$) but not for Kd, A, B or C ($P>0.27$) (Table 6). EDDM at Brandon (46.4%) was significantly less than at Saskatoon (KCRF) (50.0%). Conversely RUDM at Saskatoon (KCRF) (50.0%) was significantly lower than for Brandon (54.6%). EDDM and RUDM at Saskatoon (SF) and Wakaw did not differ from the other two sites. No genotype x site interaction ($P>0.15$) was detected for any trait.

Table 6. Brandon, Saskatoon (KCRF and SF) and Wakaw site in situ dry matter disappearance characteristics, 2004.

Site	Kd (%/hr)	A fraction (% DM)	B fraction (% DM)	C fraction (% DM)	EDDM (%)		RUDM (%)	
Brandon	11.4	8.9	58.0	33.2	46.4	a	53.6	b
Saskatoon (KCRF)	13.9	8.2	60.0	31.8	50.0	b	50.0	a
Saskatoon (SF)	14.3	9.4	58.9	31.8	49.4	ab	50.6	ab
Wakaw	12.1	8.5	61.1	30.4	47.7	ab	52.3	ab
Mean	12.9	8.7	59.5	31.8	48.4		51.6	
SE	1.32	0.27	2.35	2.32	0.79		0.79	

Significance level P<0.10.

Table 7. Valier and TR253 in situ dry matter disappearance characteristics, grown at four sites in 2004.

Site	Genotype	Kd (%/hr)		A fraction (% DM)		B fraction (% DM)	C fraction (% DM)	EDDM (%)	RUDM (%)
Brandon	Valier	9.0	a	8.7		62.1	29.2	46.6	53.4
	TR253	13.9	b	9.0		53.8	37.1	46.2	53.8
Mean		11.4		8.9		58.0	33.2	46.4	53.6
SE		0.8		0.3		7.9	8.0	1.2	1.2
Saskatoon (KCRF)	Valier	14.2		7.5	a	60.6	31.9	50.1	49.9
	TR253	13.5		8.8	b	59.4	31.8	49.9	50.1
Mean		13.9		8.2		60.0	31.8	50.0	50.0
SE		0.4		0.3		1.8	1.9	1.4	1.4
Saskatoon (SF)	Valier	10.9	a	9.3		63.1	27.7	49.7	50.3
	TR253	17.8	b	9.5		54.7	35.9	49.2	50.8
Mean		14.3		9.4		58.9	31.8	49.4	50.6
SE		2.6		0.5		4.9	4.6	1.4	1.4
Wakaw	Valier	12.2		8.6		64.1	27.2	49.0	51.0
	TR253	12.1		8.3		58.2	33.6	46.3	53.7
Mean		12.1		8.5		61.1	30.4	47.7	52.3
SE		3.8		0.9		9.5	9.2	1.5	1.5

Significance level P<0.10.

Individual site examination revealed significant differences between genotypes not identified using factorial evaluation. Kd differed significantly between genotypes at Brandon (P<0.01) and Saskatoon (SF) (P<0.08) but not at Saskatoon (KCRF) (P=0.18) or Wakaw (P=0.99). Kd was lower for Valier than TR253 at Brandon (9.0% versus

13.9%, respectively) and Saskatoon (SF) (10.9% versus 17.8%, respectively) (Table 3.6). Valier (7.49%) had significantly less A-fraction than TR253 (8.83%) at Saskatoon (KCRF) ($P<0.03$) but not at Brandon, Saskatoon (SF) or Wakaw ($P>0.33$) (Table 7). No differences in B, C, EDDM or RUDM were detected between genotypes at any site ($P>0.13$).

Discussion

As expected since locations were selected for differences in SKCS hardness between the two genotypes, SKCS hardness results showed Valier as harder than TR253. Though differing in hardness, Valier and TR253 protein levels were similar. Valier and TR253 feed particle size was also similar, contrary to the relationship between grain hardness and particle size in wheat, where softer wheat produces flour with a smaller particle size. Feed particle size measurement after coarse rolling may not be sensitive enough to detect the subtle differences in grain hardness identified by the SKCS. The SKCS hardness calibration is based on percentage of flour particles remaining above a 155 and 470-μm sieve. In this study, feed particle size was measured on considerably larger sieves, ranging from 1000 to 3350-μm.

SEM endosperm examination revealed ultrastructure differences between Valier and TR253. At three of four sites, Valier had more starch-associated protein and a compact structure in central endosperm regions versus TR253, which had a mealier structure with limited starch-associated protein.

Middle endosperm areas of Valier and TR253 however were variable, with apparent environmental influences. At Brandon and Wakaw, Valier had extensive starch-protein association compared to TR253. However, at Saskatoon (KCRF) the opposite was noted with TR253 having more starch-protein association and at Saskatoon (SF) no differences being detected. Of note, Brandon had the lowest mean protein levels of the four sites with both genotypes having low protein. Nonetheless, Valier from Brandon appeared to have the most starch-associated protein and intact protein sheets of all samples. Additionally, TR253 had more dislodged B-type starch granules compared to Valier.

In situ examination of DMDR revealed conflicting results. Factorial analysis of genotype and site effects resulted in no difference in Kd between Valier and TR253 and no genotype by site interaction. However, analysis of individual sites showed Valier had significantly slower DMDR than TR253 at Brandon and Saskatoon (SF) but not at Saskatoon (KCRF) or Wakaw. High residual error may have masked genotypic differences when analyzed factorially.

Ultrastructural differences between Valier and TR253 endosperm do not explain the in situ results. At Brandon, the tight starch-protein matrix in Valier may have had retarded degradation through delayed rumen microbial access to starch granules. However at Saskatoon (SF), Valier was also slower degrading but without the remarkable starch-protein association identified at Brandon. At Saskatoon (KCRF), TR253 had more

starch-associated protein than Valier but there was no difference in Kd. In situ variability was greater at Wakaw and may have masked potential genotype differences.

The current study did not establish a clear connection between SKCS hardness and DMDR.

From the current study, it can be concluded that, while genotypic differences exist in grain hardness between Valier and TR253, these did not translate into consistent differences in DMDR. As a result, there was no logic to pursuit of further examination of DMDR in the Valier x TR253 DH population.

From a germplasm development viewpoint, while not entirely abandoning the significant amount of Valier derived germplasm developed during the project, emphasis shifted to working more strongly with our other putative slow DMD parent genotypes WC795 and WC1064, recognizing the more difficult nature of such since both are six rowed and hulless and our primary goal is development of high quality slow DMD two row hulled germplasm.

Milling Energy and SKCS Grain Hardness

As reported previously, selected samples from the 2003 multi-site BarVYT and Bulk Increases at Saskatoon were sent to the Scottish Crop Research Institute (SCRI) in the UK where colleagues evaluated these materials using methodology which measures the energy required to mill barley samples. The results were very encouraging in that mean milling energy values for Valier, CDC Helgason and TR253 from the 2003 BarVYT were 658, 612 and 589 (LSD = 11.5) respectively indicating that, as expected, Valier required more energy to mill. Also of note were the very high milling energy requirements for the two alternative slow DMD genotypes being evaluated, WC795 and WC1064, at 785 and 700 units respectively.

Based on these encouraging results we contracted SCRI to evaluate all 247 doubled haploids from the Valier x TR253 cross along with five replicates of each check variety Valier, TR253, CDC Dolly, AC Metcalfe and CDC Helgason. This test indicated that Valier and CDC Dolly required more energy for milling than the other varieties while CDC Bold, CDC Helgason and TR253 required less. Values for Valier and TR253 were 643 and 598 joules respectively. Of note CDC Dolly was highest at 681 joules and CDC Bold was lowest at 574 joules. Comparative SKCS hardness values for these same checks were ranked in a similar fashion with Valier hardest at 59 units and TR253 softest at 53 units. The correlation between SKCS hardness and milling energy for these checks was encouraging at 0.70 suggesting a reasonable relationship between these methods of evaluation and encouraging us that these two different methodologies of evaluating physical differences between grain samples were indicative that consistent differences exist.

As part of agreement with our SCRI collaborators in 2005 we had milling energy data collected again on the same 247 doubled haploids as grown at Saskatoon in 2004. This

allowed evaluation of the heritability of SKCS hardness in this population and further evaluation of the comparison between milling energy and SKCS hardness. We also negotiated with the SCRI in 2005 to determine milling energy for samples from the 2004 BarVYT and 2004 Bulk Increases to provide more information re other varieties such as CDC Dolly and Champion (TR710) which appear to be as hard or harder than Valier and also to further evaluate WC795 and WC1064.

Results from the evaluation of the 247 doubled haploids showed that both milling energy and SKCS hardness were normally distributed. Milling energy ranged from 505 to 695 joules, considerably wider than would be expected based on parental values at 598 and 643, although there were very few samples greater than the upper parent Valier. SKCS hardness ranged from 40 to 67 units, again a much wider range than would be expected from the parents which were at 53 and 59 units. These results are encouraging in that there is a wide range available for selection and it appears that transgressive segregation exists for both measures, but are somewhat discouraging in that the data suggests these traits are inherited in a complex fashion meaning selection strategies would be complicated and difficult. Also disappointing was the fact that the correlation between milling energy and SKCS hardness for this larger, more variable sample set was only 0.43 indicating that these two measures of physical quality differences are not as closely related as indicated by the data collected from the six check cultivars.

We previously reported that during the Jan – April 2004 period Ms. Camm would conduct GPSA on samples available from the 2002 and 2003 growing seasons, albeit that the 2002 samples had been compromised from very poor weather during the 2002 season and at harvest. However, as reported above, due to our inability to repeat results of previous similar evaluations with check samples and since our experimental samples are of limited quantity, that work was suspended pending the results of the in-vivo tests. With sound samples available from the 2003 and 2004 seasons Ms. Camm concentrated on completing evaluation of those for GPSA. All samples from the 2003 and 2004 BarVYT from all sites were evaluated at two roller gap settings giving over-processed and minimally processed samples. This work required more methodology development as it appeared that grain tempering might be necessary to maximize measurement of differences. Experiments to evaluate tempering effects were conducted and it was concluded that tempering was not necessary to adequately differentiate genotypes.
Insert GC data here

During 2005 Ms Camm also evaluated parent samples and checks for other short cut screening methods and various grain quality traits including SKCS, beta glucan, protein and rate of water uptake. In addition, while plans had suggested the possibility of evaluation of grain samples for the different starch-protein matrix characteristics demonstrated by Valier using light microscopy and differential starch-protein staining in conjunction with the Grain Research Lab, Canadian Grain Commission to attempt to mimic the differences previously reported from scanning electron microscopy work, this collaboration was not possible. In lieu of that during 2006 a test was developed locally to evaluate light reflectance from grain endosperm using light microscopy at the CDC to attempt to differentiate between grain types. Insert GC data In addition, as described

above, Scanning Electron Microscopy was again used during 2005 to visualize and rationalize the inconsistency of differences in DMD rate expressed by Valier and TR253 samples evaluated from different environments in the in-vivo bovine experiments. Finally, working with advice and guidance from Ag Bioresource Engineering, U of S, we attempted to determine energy required to roll the various grain samples from our trials using our in-house roller in anticipation there would be a differential between slow DMD versus normal types. Unfortunately excess baseline fluctuation in power measurement meant this technique was not useable.

Results

SKCS hardness of genotypes ranged from 38.2 to 56.1 (Table 8). McLeod was hardest, followed by Valier and CDC Dolly. CDC Trey, Newdale, TR253, CDC Helgason, and CDC Bold followed, with CDC Bold the softest. SKCS hardness ranged from 35.2 to 55.8 across environments (data not shown). Milling energy of genotypes ranged from 617 to 736 joules (Table 8). McLeod and CDC Dolly required significantly more energy to mill, followed by Valier, Newdale, CDC Helgason, Trey and TR253. CDC Bold required the least energy to mill, indicating a softer endosperm. Milling energy ranged from 629 to 701 joules across environments (data not shown). Light reflectance ranged from 19.2 to 23.8 thousand pixels/mm^2 across genotypes (Table 8). McLeod was hardest (steeliest), with Valier, CDC Dolly and CDC Trey following. CDC Helgason, Newdale and TR253 were softer, with CDC Bold being the softest (mealiest). Light reflectance ranged from 20.0 to 24.5 thousand pixels/mm^2 across environments (data not shown).

Table 8. Mean Grain hardness, protein, beta-glucan, SKCS seed weight, diameter and moisture of eight Western Canadian barley cultivars across 12 sites, 2003 and 2004.

Cultivar	Type	SKCS Hardness	Milling Energy (j)	Light Reflectance (1000 pixels/mm^2)	% Protein	% Beta-glucan	SKCS Seed		
							Weight (g)	Diameter (mm)	Moisture (%)
McLeod	Feed	56.1 a	736 a	19.2 a	12.3 a	3.98 cd	51.6 ab	2.62	10.3
Valier	Feed	50.4 b	691 b	21.1 b	11.9 ab	4.35 b	48.0 c	2.57	10.2
CDC Dolly	Feed	48.4 bc	733 a	21.4 bc	12.0 ab	4.72 a	51.5 ab	2.64	10.3
CDC Trey	Feed	47.1 cd	645 d	21.6 bc	11.1 c	3.88 d	50.4 b	2.58	10.3
Newdale	Malt	45.4 cd	675 bc	22.5 cde	11.9 ab	4.12 c	50.3 b	2.57	10.4
TR253	Malt	44.8 d	644 d	23.6 de	11.5 bc	4.01 cd	48.5 c	2.59	10.1
CDC Helgason	Feed	44.4 d	663 cd	22.3 bcd	11.8 abc	3.88 d	48.3 c	2.52	10.1
CDC Bold	Feed	38.2 e	617 e	23.8 e	11.4 bc	3.40 e	52.8 a	2.54	10.4
Mean		**46.9**	**676**	**21.9**	**11.7**	**4.04**	**50.2**	**2.58**	**10.2**
S.E.		**0.7**	**5.3**	**0.3**	**0.2**	**0.04**	**0.4**	**0.01**	**0.1**

Protein content ranged from 11.1 to 12.3% (Table 8) with McLeod, CDC Dolly, Valier, Newdale and CDC Helgason highest followed by TR253, CDC Bold and CDC Trey. Protein content varied from 8.9% to 15.5% across environments (data not shown). Percent beta-glucan of genotypes ranged from 3.4% to 4.7% (Table 8). CDC Dolly had most followed by Valier, Newdale, TR253 and McLeod. CDC Helgason, CDC Trey and CDC Bold followed, with CDC Bold having the lowest. Beta-glucan varied from 3.8% to 4.3% across environments (data not shown).

SKCS seed weight of genotypes ranged from 48.0 to 52.8 grams (Table 8). CDC Bold, McLeod and CDC Dolly had the largest seed, followed by CDC Trey and Newdale. TR253, CDC Helgason and Valier had the smallest seeds. SKCS seed weight varied from 44.0 to 56.2 grams across environments (data not shown). No difference between genotypes was detected for SKCS diameter or moisture.

Table 9. Feed particle size analysis of eight Western Canadian barley cultivars across twelve sites (2003 and 2004), processing methods combined.

Cultivar	Type	% Particles					
		3.35-mm	2.36-mm	2.00-mm	1.40-mm	1.00-mm	<1.00-mm
Newdale	Malt	15.6 a	38. bc 7	19.1 d	17.2 abc	4.62 bc	4.86 cd
McLeod	Feed	14.9 ab	39. abc 5	19.5 cd	16.4 cd	4.52 c	5.16 bc
CDC Dolly	Feed	14.0 abc	41. a 2	19.8 bcd	16.0 d	4.22 d	4.82 d
CDC Helgason	Feed	13.2 bcd	39. abc 5	20.7 bc	17.1 abc	4.58 bc	4.91 cd
CDC Trey	Feed	12.5 cd	39. abc 5	20.2 bcd	17.4 abc	4.98 a	5.40 ab
TR253	Malt	12.1 cd	38. bc 9	21.0 ab	18.1 ab	4.85 ab	5.03 cd
Valier	Feed	12.1 cd	40. abc 7	20.7 bc	17.1 bc	4.59 bc	4.87 cd
CDC Bold	Feed	11.5 d	37. c 6	22.1 a	18.2 a	5.11 a	5.64 a
Mean		13.2	39. 5	20.4	17.2	4.69	5.09
S.E.		0.5	0.5	0.3	0.2	0.07	0.07

3.35-mm Particle size of genotypes ranged from 11.5 to 15.6% (Table 9). Newdale, McLeod, and CDC Dolly had the highest percentage, followed by CDC Helgason, CDC Trey, TR253 and Valier. CDC Bold had the lowest percentage 3.35-mm particles. 3.35-mm particle size varied from 10.5 to 19.5% across environments (data not shown).

Genotype 2.36-mm particle size ranged from 37.6 to 41.2%, with CDC Dolly, Valier, McLeod, CDC Helgason, and CDC Trey having the largest percentage (Table 9).

TR253, Newdale and CDC Bold followed with CDC Bold having the lowest. 2.36-mm particle size varied from 37.7 to 43.5 across environments (data not shown).

2.00-mm Particle size of genotypes ranged from 19.1 to 22.1% (Table 9). CDC Bold and TR253 had the most 2.00-mm particles with CDC Helgason, Valier, CDC Trey and CDC Dolly following. McLeod and Newdale had the lowest percentage of this fraction. Environments varied from 18.4 to 22.2% in 2.00-mm particle size (data not shown).

Genotype 1.40-mm particle size ranged from 16.0 to 18.2%, with CDC Bold, TR253, CDC Trey, Newdale and CDC Helgason having the largest percentages (Table 9). Valier, McLeod and CDC Dolly had the least of this fraction. 1.40-mm particle size varied from 14.8 to 18.7% across environments (data not shown).

1.00-mm Particle size of genotypes ranged from 4.22 to 5.11% (Table 9). CDC Bold, CDC Trey and TR253 had the largest percentage of this size, followed by Newdale, Valier, CDC Helgason, McLeod and CDC Dolly. CDC Dolly had the least 1.00-mm particles. Environments varied from 3.85 to 5.06% for this particle size (data not shown).

<1.00-mm Particle size of genotypes ranged from 4.82 to 5.64% (Table 9). CDC Bold and CDC Trey had the largest percentage of this fraction, followed by McLeod, TR253, CDC Helgason, Valier, Newdale, and CDC Dolly. CDC Dolly had the least particles <1.00-mm. Environments varied from 3.91 to 5.94% for particles <1.00-mm (data not shown).

Table 10. Mean, large, mid-sized, and fine feed particle size of eight Western Canadian barley cultivars across twelve sites (2003 and 2004), processing methods combined.

Cultivar	Type	Mean Particle Size (μm)	% Large Particles >2.36-mm	% Mid-sized Particles 1.40-2.36-mm	% Fine Particles <1.40-mm
Newdale	Malt	2465 a	54.3 ab	36.2 cde	9.48 cd
McLeod	Feed	2457 ab	54.4 ab	35.9 de	9.68 c
CDC Dolly	Feed	2450 ab	55.2 a	35.7 e	9.03 d
CDC Helgason	Feed	2403 abc	52.7 bc	37.8 bc	9.49 cd
Valier	Feed	2379 bcd	52.8 bc	37.7 bc	9.47 cd
CDC Trey	Feed	2358 cd	52.0 c	37.6 bcd	10.39 ab
TR253	Malt	2350 cd	51.1 cd	39.0 ab	9.88 bc
CDC Bold	Feed	2307 d	49.0 d	40.2 a	10.75 a
Mean		2396	52.7	37.5	9.77
S.E.		20	0.5	0.4	0.13

Mean particle size of genotypes ranged from 2307 to 2465-μm (Table 10). Newdale, McLeod, CDC Dolly and CDC Helgason had the largest mean particle size, with Valier, CDC Trey, TR253 and CDC Bold following. CDC Bold had the smallest mean particle size. Mean particle size varied from 2290 to 2645-μm across environments (data not shown).

Percent large particles (>2.36-mm) of genotypes varied from 49.0 to 55.2, with CDC Dolly, McLeod and Newdale having the largest percentages (Table 10). Valier, CDC Helgason, CDC Trey, TR253 and CDC Bold followed, with CDC Bold having the lowest % large particles. Percent large particles (>2.36-mm) ranged from 49.3 to 57.9% across environments (data not shown).

Genotype percent mid-sized particles (1.40-2.36-mm) ranged from 35.7 to 40.2% (Table 10). CDC Bold and TR253 had the largest percentage, followed by CDC Helgason, Valier, CDC Trey, Newdale, McLeod with CDC Dolly having the lowest percentage. Mid-sized particles varied from 35.1 to 40.6% across environments (data not shown).

Genotype percent fine particles (<1.40-mm) ranged from 9.0 to 10.8% (Table 10). CDC Bold and CDC Trey had the most fine-particles. TR253, McLeod, CDC Helgason, Newdale, Valier followed. CDC Dolly had the least fine particles. Fine particles (<1.40-mm) varied from 7.8 to 11.0 across environments (data not shown).

Table 11. Pearson correlation analysis of grain hardness, protein, beta-glucan, mean particle size, and large, mid-sized and fine particles (N=8).

	Milling Energy	SKCS Hardness	Light Reflect	% Protein	% Beta-glucan	SKCS Weight	MPS (All RG)	MPS (1.3-mm RG)	MPS (1.7-mm RG)	Large Particles (All RG)	Mid-sized Particles (All RG)	Fine Particles (All RG)
Milling Energy	1											
SKCS Hardness	0.84 *P<0.009*	1										
Light Reflectance	-0.83 *P<0.01*	-0.94 *P<0.0005*	1									
% Protein	0.86 *P<0.006*	0.65 *P=0.08*	-0.65 *P=0.08*	1								
% Beta-glucan	0.75 *P<0.03*	0.54 *P=0.17*	-0.43 *P=0.29*	0.50 *P=0.20*	1							
SKCS Weight	0.09 *P=0.83*	-0.11 *P=0.80*	-0.07 *P=0.87*	0.08 *P=0.86*	-0.25 *P=0.55*	1						
MPS (All RG)	0.83 *P<0.01*	0.65 *P=0.08*	-0.63 *P=0.09*	0.78 *P<0.02*	0.63 *P=0.10*	0.05 *P=0.91*	1					
MPS (1.3-mm RG)	0.88 *P<0.004*	0.69 *P=0.06*	-0.65 *P=0.08*	0.69 *P=0.06*	0.85 *P<0.007*	-0.04 *P=0.91*	0.93 *0.0008*	1				

MPS (1.7-mm RG)	0.77 *P<0.02*	0.61 *P=0.11*	-0.60 *P=0.12*	0.79 *P<0.02*	0.52 *P=0.19*	0.08 *P=0.85*	0.99 *<0.0001*	0.86 *P<0.005*	1			
Large Particles (All RG)	0.89 *P<0.003*	0.73 *P<0.04*	-0.70 *P=0.05*	0.72 *P<0.04*	0.80 *P<0.02*	-0.04 *P=0.92*	0.96 *P<0.0002*	0.99 *P<0.0001*	0.91 *P<0.002*	1		
Mid-sized Particles (All RG)	-0.87 *P<0.005*	-0.75 *P<0.03*	0.75 *P<0.03*	-0.67 *P=0.07*	-0.72 *P<0.05*	-0.07 *P=0.86*	-0.95 *P<0.0004*	-0.97 *P<0.0001*	-0.91 *P<0.002*	-0.98 *P<0.0001*	1	
Fine Particles (All RG)	-0.77 *P<0.02*	-0.52 *P=0.18*	0.44 *P=0.28*	-0.72 *P<0.04*	-0.87 *P<0.005*	0.37 *P=0.37*	-0.79 *P<0.02*	-0.87 *P<0.004*	-0.74 *P<0.04*	-0.86 *P<0.007*	-0.75 *P<0.03*	1

MPS = mean particle size
1.3-mm RG = roller gap size 1.3-mm
1.7-mm RG = roller gap size 1.7-mm
All RG = combined means of processing methods

Correlation analysis is summarized in Table 11. Milling energy was positively correlated (n=8) with SKCS hardness (r = 0.84, P<0.009) (Figure 3.1), protein (r = 0.86, P<0.006) (Figure 3.2), and beta-glucan (r = 0.75, P<0.03) (Figure 3.3). However, the milling energy versus beta-glucan correlation was non-significant (P=0.57, n=6) when high (CDC Dolly) and low (CDC Bold) beta-glucan genotypes were removed from the analysis. In contrast, the milling energy versus beta-glucan correlation was stronger (r = 0.96, P<0.0005, n=7) when the high protein genotype McLeod was removed. Effects of the small genotype sample size was evident throughout the study for some trait interrelationships.

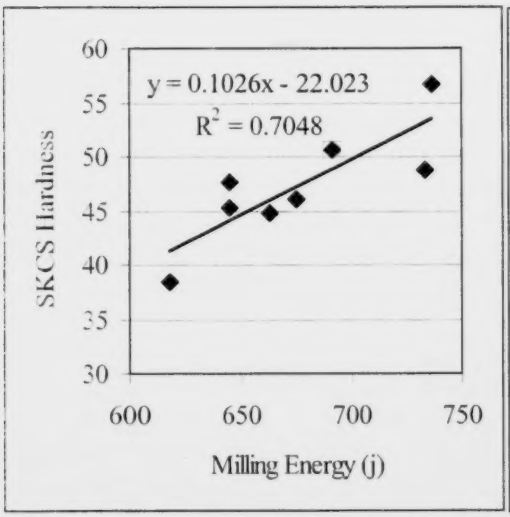

Figure 3.1. Relationship between SKCS hardness and milling energy for eight barley genotypes.

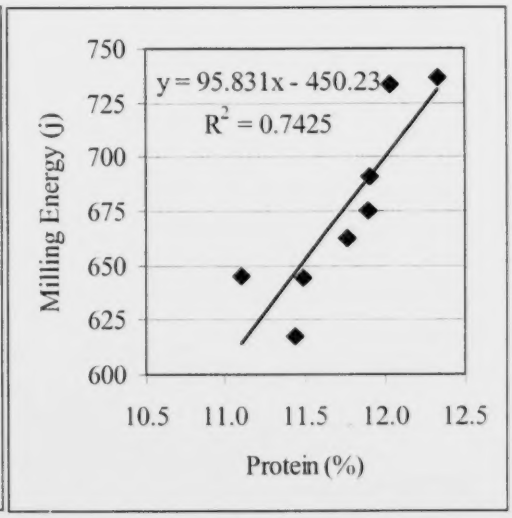

Figure 3.2. Relationship between milling energy and protein for eight barley genotypes.

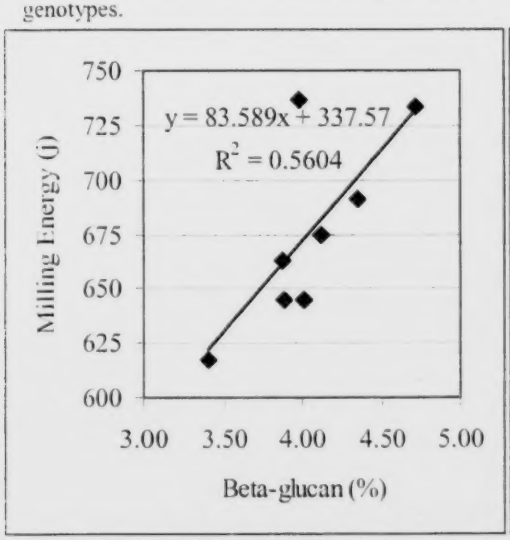

Figure 3.3. Relationship between milling energy and beta-glucan for eight barley genotypes.

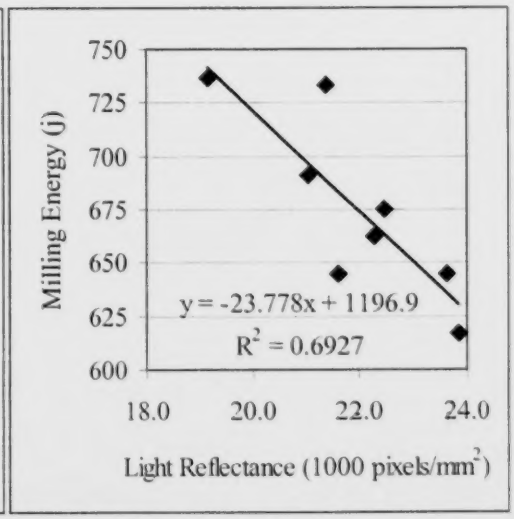

Figure 3.4. Relationship between milling energy and light reflectance for eight barley genotypes.

Both milling energy (r = -0.83, P<0.01) (Figure 3.4) and SKCS hardness (r = -0.94, P<0.0005) (Figure 3.5) were negatively correlated with light reflectance. SKCS hardness and protein

(P=0.08), SKCS hardness and beta-glucan (P=0.17), light reflectance and protein (P=0.08) and light reflectance and beta-glucan (P=0.29) were not significantly correlated. However, the SKCS hardness versus protein correlation was significant (r = 0.80, P<0.05, n=6) when high (McLeod) and low (CDC Trey) protein genotypes were removed from the analysis. Similar results were found for light reflectance and protein (r = -0.92, P<0.0009, n=6).

Mean particle size from combined processing methods (ALL RG) was positively correlated with milling energy (r = 0.83, P<0.01) (Figure 3.6) and protein (r = 0.78, P<0.02) (Figure 3.7). Separate analysis of processing methods showed mean particle size of over-processed material (1.3-mm RG) positively correlated with beta-glucan (r = 0.85, P<0.007). In contrast, mean particle size of minimally processed material (1.7-mm RG) was positively correlated with protein (r = 0.79, P<0.02) rather than beta-glucan (P=0.19). However, mean particle size and protein/beta-glucan correlations were non-significant (P=0.08, n=6) when high (CDC Dolly) and low (CDC Bold) beta- glucan genotypes were removed from the analysis. Contrarily, mean particle size and protein/beta-glucan correlations became more significant when high (McLeod) and low (CDC Trey) protein genotypes were removed from the analysis. Mean particle size (over-processed) was positively correlated with protein (r = 0.93, P<0.007, n=6) and beta-glucan (r = 0.90, P<0.01, n=6). Mean particle size (minimally processed) remained positively correlated with protein (r = 0.81, P<0.05, n=6) but not beta-glucan (P=0.20).

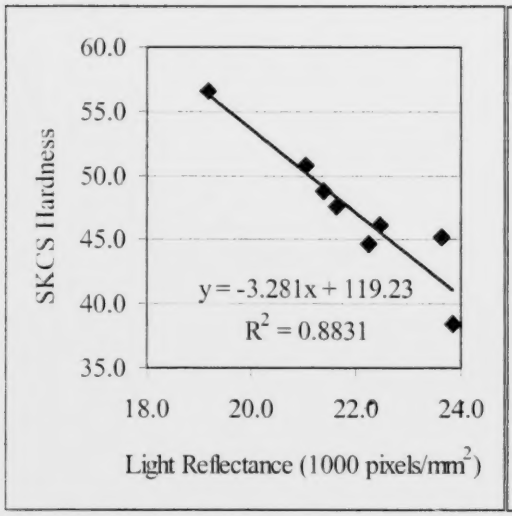

Figure 3.5. Relationship between SKCS hardness and light reflectance for eight barley genotypes.

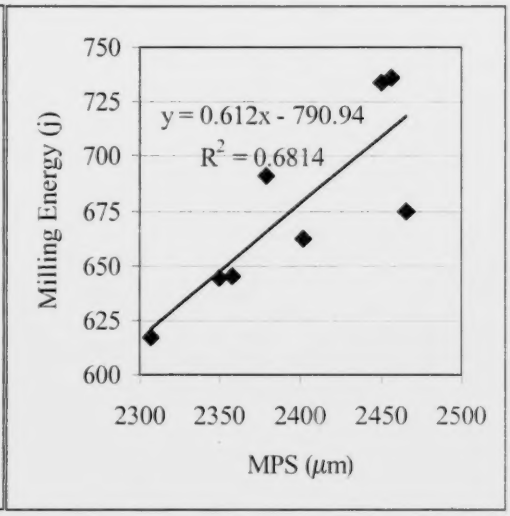

Figure 3.6. Relationship between milling energy and mean particle size (ALL RG) for eight barley genotypes.

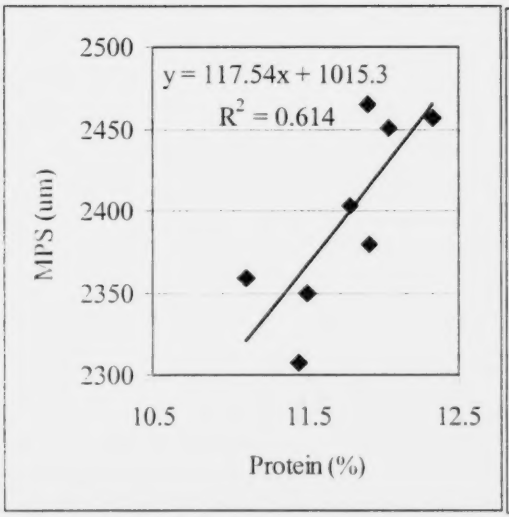

Figure 3.7. Relationship between MPS (ALL RG) and protein for eight barley genotypes.

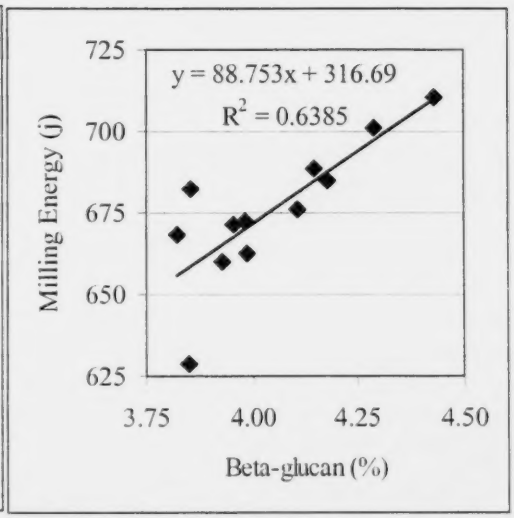

Figure 3.8. Relationship between milling energy (j) and beta-glucan across twelve environments.

From combined processing methods (ALL RG), percent large particles was positively correlated with milling energy ($r = 0.89$, $P<0.003$), SKCS hardness ($r = 0.73$, $P<0.04$), protein ($r = 0.72$, $P<0.04$) and beta-glucan ($r = 0.80$, $P<0.02$). Percent mid-sized particles was negatively correlated with milling energy ($r = -0.87$, $P<0.005$), SKCS hardness ($r = -0.75$, $P<0.03$), and beta-glucan ($r = -0.72$, $P<0.05$) while positively correlated with light reflectance ($r = 0.75$, $P<0.03$). Percent fine particles was negatively correlated with milling energy ($r = -0.77$, $P<0.02$), protein ($r = -0.72$, $P<0.04$) and beta-glucan ($r = -0.87$, $P<0.005$) only.

To evaluate environmental influences on grain hardness, Pearson's correlation analysis was performed on protein, beta-glucan and hardness site means. Milling energy, SKCS hardness, light reflectance and mean particle size (ALL RG) were not correlated with protein levels across the twelve environments ($P>0.25$). Similarly, SKCS hardness and mean particle size (ALL RG) were not correlated with beta-glucan levels ($P>0.05$, $n=12$). However, milling energy was positively correlated with beta-glucan levels ($r = 0.80$, $P<0.002$) (Figure 4.8) across the twelve environments, while light reflectance/beta-glucan showed a negative trend ($r = -0.57$, $P=0.05$, $n=12$).

Discussion

Genotypes differed in SKCS hardness, milling energy and endosperm light reflectance with McLeod, CDC Dolly and Valier being consistently harder and CDC Bold softest. Hardness ranking of genotypes based on feed particle size differed slightly with Newdale, McLeod and CDC Dolly having larger particle size and CDC Bold small. Hardness methodology ranked genotypes similarly across environments, although levels varied at each. The exception in the current study was Valier and TR253 as large differences between these genotypes in 2003 were minimal in 2004 (data not shown).

Although SKCS hardness, milling energy and endosperm light reflectance methods correlated well, only milling energy showed a consistent relationship with feed particle size. Both milling energy and feed particle size measurements are products of shearing action from roller milling, whereas SKCS hardness is based on compression force. In has been reported for wheat that the crushed grain after SKCS analysis contained larger particles compared to roller milling. This could explain the current results in which SKCS hardness (and light reflectance) was correlated with large and mid-sized particles but not fine particles or mean particle size. Light reflectance may also relate to compression rather than shearing.

Milling energy appeared to be influenced by the protein and beta-glucan of genotypes, with harder grain having greater levels of both.

SKCS hardness and light reflectance were not correlated with protein or beta-glucan. Light reflectance was measured using single transverse sections of multiple grains, whereas protein and beta-glucan were analyzed on blended whole grain samples. Therefore, protein and/or beta-glucan could be influencing light reflectance in the observed section without correlation with the whole grain measurement.

Feed particle size may also be influenced by protein and beta-glucan content. Higher protein genotypes tended to remain in larger pieces with minimal processing compared to lower protein genotypes. This relationship was not evident with over-processed barley, where conversely beta-glucan influenced particle size. A plausible explanation is that with minimal roller force, an increased level of starch-associated protein maintained cohesiveness of the endosperm cellular structure, producing larger particles. However, a larger force caused the starch-protein matrix to shear, relying on the beta-glucan in the cell walls to maintain endosperm integrity.

Work from Montana suggested that increased mean particle size of barley reduced DMDR in cattle. In the current study, larger mean particle size was associated with increased grain protein and/or beta-glucan. Breeding for higher protein and beta-glucan may increase mean particle size of barley and indirectly reduce DMDR in cattle.

Milling energy may be a useful tool to identify barley with increased mean particle size. Milling energy is a quick method of evaluation that requires only one minute per sample and five grams of seed. It would also allow earlier generation selection for this trait. Feed particle size analysis requires significantly more seed (50-grams) and time, with rolling and particle separation a two-step process.

In the current study, Valier and TR253 were significantly different for SKCS hardness, milling energy, light reflectance and beta-glucan. However, there were no differences in feed particle size. It was concluded that sufficient differences existed in grain hardness to evaluate Valier x TR253 DH population for SKCS hardness, milling energy and endosperm light reflectance but not feed particle size.

Heritability of SKCS Hardness

As indicated earlier, as part of agreement with our SCRI collaborators in 2005 we had milling energy data collected again on the same 247 doubled haploids as grown at Saskatoon in 2004. This allowed evaluation of the heritability of SKCS hardness in this population and further evaluation of the comparison between milling energy and SKCS hardness.

Results

Valier and TR253 differed in SKCS hardness, milling energy, light reflectance, protein and beta-glucan (Table 12). Valier was harder than TR253, as measured by SKCS hardness, milling energy and light reflectance and had higher protein and beta-glucan. No differences were detected between Valier and TR253 for SKCS seed weight, diameter or moisture. No further analysis was performed for these three SKCS measured seed traits.

Table 12. Valier and TR253 mean grain hardness, protein, beta-glucan (2004 only) and SKCS seed weight, diameter, and moisture characteristics, grown in 2003 and 2004.

Genotype	SKCS Hardness	Milling Energy (j)	Light Reflectance (1000 pixels/mm^2)	Protein (%)	Beta-glucan (%)	SKCS Seed		
						Weight (g)	Diameter (mm)	Moisture (%)
Valier	56.2 a	672 a	15.8 b	12.1 a	4.47 a	50.6	2.76	9.88
TR253	51.9 b	629 b	18.3 a	11.4 b	4.01 b	50.2	2.74	9.93
Mean	54.0	651	17.0	11.7	4.24	50.4	2.75	9.90
SE	0.40	5.5	0.20	0.09	0.06	0.20	0.01	0.03

Significance level P<0.05.

SKCS hardness, milling energy, and light reflectance differed between years (P<0.03), although ranking of Valier and TR253 did not change (data not shown). The protein level of Valier was similar across years (P>0.07) but TR253 protein was higher in 2003 compared to 2004 (P<0.03). It is not known if beta-glucan varied across years due to limited data availability (2004 only).

Analysis of variance revealed significant differences between genotypes for SKCS hardness, milling energy, light reflectance, protein and beta-glucan (P<0.0001). Significant differences between years also existed for SKCS hardness, light reflectance and protein (P<0.0002). It is not known if milling energy and beta-glucan differed between years, due to limited data availability. No genotype by year interaction was detected for light reflectance or protein (P≥0.05). Non-cross over genotype by year interaction was detected for SKCS hardness (P<0.0001). It is not known if genotype by year interactions affected milling energy and beta-glucan, due to limited data availability. All years/reps were combined for final analysis of grain hardness, protein and beta-glucan.

The population ranged in SKCS hardness from 42.5 to 61.5 with a mean of 52.9 (Table 5.2), milling energy from 558 to 716 joules with a mean of 640 joules, and light reflectance from 14.0 to 20.3 thousand pixels/mm^2 with a mean of 16.8 thousand pixels/mm^2. Protein varied from 10.8

to 13.5% with a mean of 12.0%, while beta-glucan varied from 3.14 to 5.02% with a mean of 4.09% (Table 13).

Table 13. SKCS hardness, milling energy, light reflectance, protein and beta-glucan midparent values and population description for the Valier/TR253 DH population.

Trait	Midparent value	Population mean	Range	Min	Max	SE	W	Pr<W
SKCS Hardness	54.0	52.9	19.0	42.5	61.5	0.21	0.993	0.34
Milling Energy (j)	651	640	158	558	716	1.70	0.996	0.71
Light Reflectance (1000 pixels/mm^2)	17.0	16.8	6.4	14.0	20.3	0.10	0.974	0.046
Protein (%)	11.7	12.0	2.7	10.8	13.5	0.03	0.993	0.27
Beta-glucan (%)	4.24	4.09	1.88	3.14	5.02	0.02	0.997	0.92

Shapiro-Wilk's (W) test for normality (Table 13) indicated the population was normally distributed (P>0.27) for SKCS hardness (Figure 4.1), milling energy (Figure 4.2), protein (Figure 4.3) and beta-glucan (Figure 4.4). However, light reflectance (Figure 4.5) was slightly leptokurtic, thus not normally distributed (P<0.046).

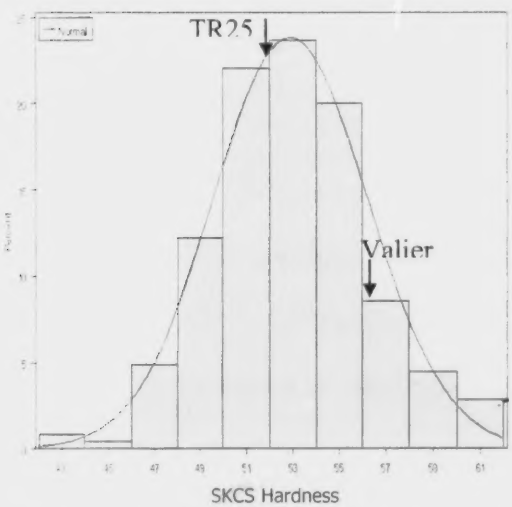

Figure 4.1. SKCS hardness distribution of Valier/TR253 DH population (n=245), grown in 2003 and 2004.

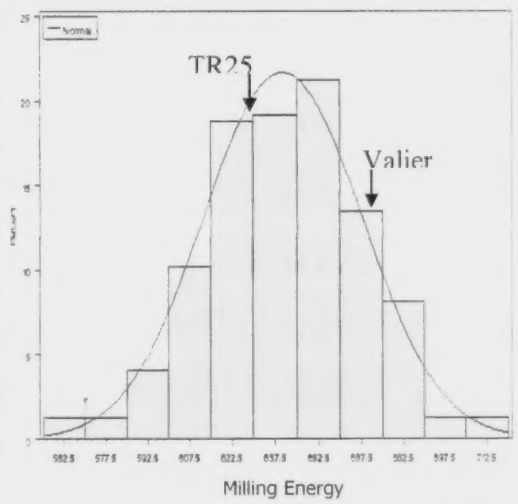

Figure 4.2. Milling energy distribution of Valier/TR253 DH population (n=245), grown in 2003 and 2004.

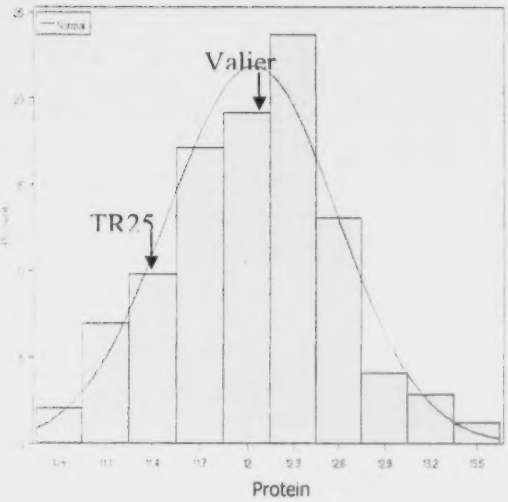

Figure 4.3. Protein distribution of Valier/TR253 DH population (n=245), grown in 2003 and 2004.

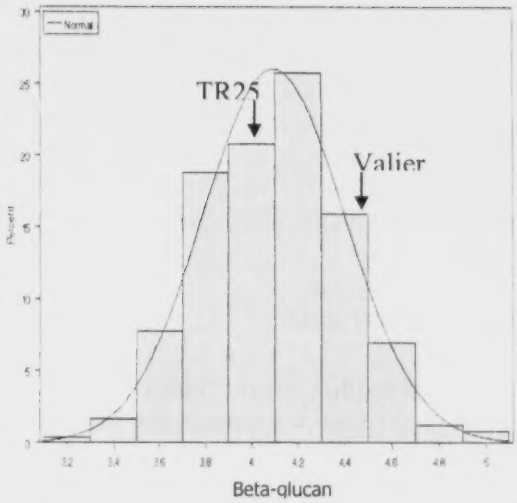

Figure 4.4. Beta-glucan distribution of Valier/TR253 DH population (n=245), grown in 2004.

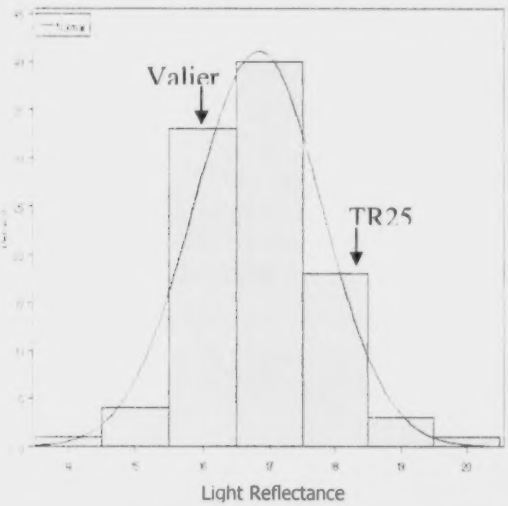

Figure 4.5. Light reflectance distribution of Valier/TR253 DH population (n=100), grown in 2003 and 2004.

Recombination of genes in the population resulted in transgressive segregation for SKCS hardness and milling energy, with more segregates softer than soft parent TR253. However, light reflectance showed few progeny were harder than Valier in 2003, with none in 2004. No transgressive segregation beyond TR253 was apparent for light reflectance in any year. For protein, transgressive segregation occurred beyond the high protein Valier. Few segregants had less protein than TR253 in 2003 and none in 2004. No transgressive segregation for beta-glucan was apparent beyond high parent Valier, with few having less beta-glucan than TR253.

Table 14. SKCS hardness, light reflectance, and protein broad-sense heritability (h^2), skewness (g_1), kurtosis (g_2) coefficients for the Valier/TR253 DH population.

Trait	Heritability (H^2)	Skewness (g_1)	Kurtosis (g_2)
SKCS Hardness	0.75	0.15	0.21
Light Reflectance (1000 pixels/mm^2)	0.53	0.42*	1.77**
Protein (%)	0.41	0.06	-0.14

*Significant at 0.05 level.
**Significant at 0.01 level.

Broad-sense heritability for the Valier/TR253 DH population was estimated as 0.75 for SKCS hardness, 0.53 for light reflectance and 0.41 for protein (Table 14). Skewness (g_1) and kurtosis (g_2) coefficients were not significantly different than zero for SKCS hardness ($P>0.17$) and protein ($P>0.33$), indicating an absence of epistasis (Table 14). However, a positive g_1 result ($P<0.04$) for light reflectance suggested complementary gene interaction existed for the light reflectance loci in the DH population (Table 14). This result was further substantiated with g_2 also being significantly greater than zero for light reflectance ($P<0.0002$). Therefore, narrow-sense heritability equals broad-sense heritability for SKCS hardness and protein, but not light reflectance.

Table 15. Valier/TR253 DH population Pearson correlation analysis of SKCS hardness, milling energy, light reflectance, protein, and beta-glucan. N=245 for all analysis except light reflectance (N=100).

	SKCS Hardness	Milling Energy (j)	Light Reflectance (1000 pixels/mm^2)	Protein (%)	Beta-glucan (%)
SKCS Hardness	1				
Milling Energy (j)	**0.44**	1			
	P<0.0001				
Light Reflectance (1000 pixels/mm^2)	**-0.28**	**-0.46**	1		
	P<0.005	***P<0.0001***			
Protein (%)	**-0.17**	**0.18**	**-0.14**		
	P<0.008	***P<0.004***	***P=0.15***	1	
Beta-glucan (%)	**0.42**	**0.43**	**-0.18**	**0.22**	
	P<0.0001	***P<0.0001***	***P=0.08***	***P<0.0006***	1

Pearson's correlation analysis is summarized in Table 15. SKCS hardness (n=245) was positively correlated with milling energy (r = 0.44, P<0.0001) (Figure 5.1), and beta-glucan (r = 0.42, P<0.0001) (Figure 5.2). A minimal negative correlation was present for SKCS hardness and both light reflectance (r = -0.28, P<0.005, n=100) and protein (r = -0.17, P<0.008, n=245). Milling energy was negatively correlated with light reflectance (r = -0.46, P<0.0001, n=100) (Figure 5.3) and positively correlated with beta-glucan (r = 0.43, P<0.0001, n=245) (Figure 5.4). A minimal positive correlation was present for milling energy and protein (r = 0.18, P<0.004,

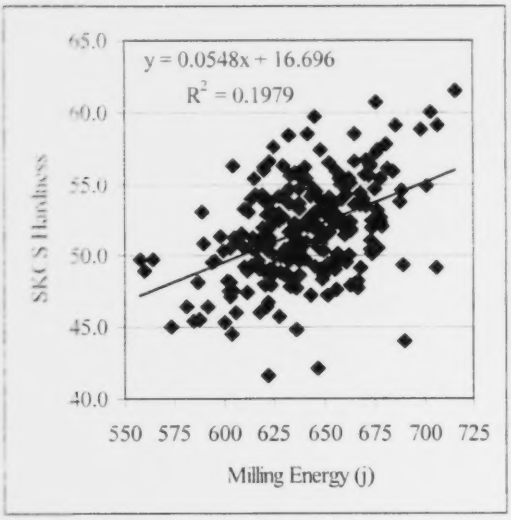

Figure 5.1. Relationship between SKCS hardness and milling energy for 245 Valier/TR253 DH genotypes.

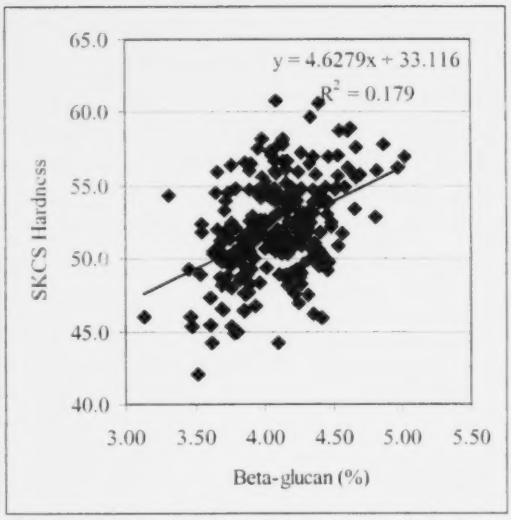

Figure 5.2 Relationship between SKCS hardness and beta-glucan for 245 Valier/TR253 DH genotypes.

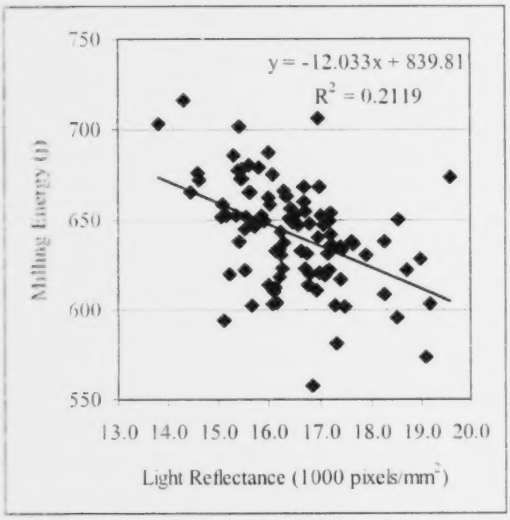

Figure 5.3. Relationship between milling energy and light reflectance for 100 Valier/TR253 DH genotypes.

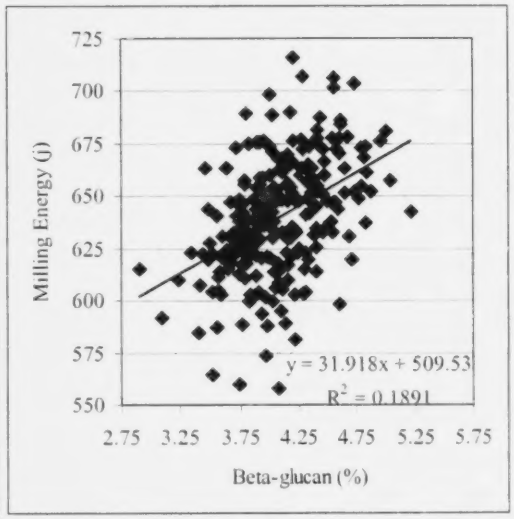

Figure 5.4. Relationship between milling energy and beta-glucan for 245 Valier/TR253 DH genotypes.

n=245). Light reflectance showed no correlation with either protein or beta-glucan ($P>0.08$, n=100).

Discussion

Similar to results reported in the genotype experiment, Valier had significantly harder grain than TR253 and had significantly more protein and beta-glucan. The DH population was normally distributed for SKCS hardness, milling energy, protein and beta-glucan, indicating quantitative inheritance. The unexpected absence of normality for light reflectance may be attributed to the smaller sampling of the population, where only 100 progeny were tested rather than 245 as with SKCS hardness and milling energy. Furthermore, the methodology may have been restricted in distinguishing smaller variations between progeny. Examination of the skewness/kurtosis would however suggest that light reflectance was influenced by complementary gene interaction between loci.

Transgressive segregation was detected for SKCS hardness, milling energy, and protein, with minor amounts for beta-glucan and light reflectance. SKCS hardness, milling energy, and protein showed transgressive segregation in both directions, suggesting that parents contributed both positive and negative alleles for these traits. No transgressive segregation was detected beyond the high beta-glucan Valier parent or the soft TR253 for light reflectance. In the current study, more softer segregants were detected than hard for SKCS hardness and milling energy, suggesting more dominant gene action for softness. In contrast to hardness, more high protein segregants were detected in the population than soft.

The SKCS hardness heritability of 0.75 was lower than the previous published range of 0.82 to 0.96, however, in the current study, the SKCS hardness population range of 42 to 62 for TR253/Valier was considerably narrower than the range of 35 to 89 in the published report for 40 diverse breeding lines and commercial varieties. The narrower range may have hindered proper determination of genetic variance, resulting in a lower estimate of heritability. However, the difference may also be related to the different techniques used to measure heritability. In the current study, 245 DH progeny from the same genetic background were evaluated for hardness, compared to 40 breeding lines selected from a diverse genetic background in the Fox study. In the current study, broad and narrow-sense heritability were equal for SKCS hardness, due to the absence of epistasis in this DH population.

Light reflectance heritability of 0.53 was lower than that for SKCS hardness, suggesting population variability was influenced similarly by environmental and genetic influences. Again, the methodology may have been restricted in distinguishing smaller variations between progeny, resulting in more experimental variance and a lower heritability estimate. In the current study, heritability was reported only as broad-sense for light reflectance, due to detection of complementary gene interaction. As mentioned previously, these results are similar to epistasis detected by Thomas et al. (1996) with milling energy.

Correlations between traits across this whole population showed some similarities to those of the varieties in the smaller genotype experiment. SKCS hardness was positively correlated with milling energy while milling energy was negatively correlated with light reflectance and positively with beta-glucan. Light reflectance again showed no association with beta-glucan. However, unlike the genotype experiment, SKCS hardness showed little association with protein

but was positively correlated with beta-glucan. Milling energy and light reflectance showed no association with protein.

In the current study, it is not known if environment affected the SKCS hardness-beta-glucan correlation due to the limited beta-glucan data availability (2004 only). However, the genotype experiment beta-glucan results had indicated non-cross over genotype by environment interactions.

The current study further substantiates the relationship between milling energy and beta-glucan, however, the milling energy-protein correlation reported in the genotype experiment was minimal in this DH population.

The current study reported that the Valier/TR253 DH population was normally distributed for SKCS hardness, milling energy, protein and beta-glucan, indicating quantitative inheritance for these traits. However, light reflectance was not normally distributed, with significant kurtosis suggesting complementary gene interactions. Transgressive segregation was detected for SKCS hardness and milling energy beyond both parents. However, fewer hard segregants may increase the difficulty of trait selection by requiring larger population sizes. Nonetheless, heritability was high for SKCS hardness, making selection for this trait feasible for breeding.

Field work Objective #1

As described below, several experiments were successfully grown and harvested during 2003 and 2004 with reasonable quantities of samples available for analyses, especially the 2003 and 2004 Barley Variety Yield Trials (BarVYT). Samples from these BarVYT trials were used to evaluate genotype x environment effects on the slow DMD trait. Results also indicated that the Valier x TR253 population was most suitable for subsequent experimentation since "hardness" differences are not as evident between Valier and CDC Helgason. Fortunately the Valier x TR253 cross was also that from which we have a large doubled haploid population providing a superior set of materials for genetic analysis thus the remaining portions of the sub-project concentrated on that population.

Also as described below in detail we have offspring from crosses with both WC795 and WC1064 (putatively even slower in DMD rate selections from the barley World Collection) for initial grain quality evaluation. SKCS data collected from samples produced in the 2003/2004 New Zealand winter nursery where RILs from three crosses were increased indicated dramatic variation for hardness with values ranging to near 90 units with normal checks at 50 units, Valier at 55 units, WC1064 at 65 units, and WC795 at 80 units (note that differences of 5 units are generally significant). Selections from these populations were grown at Saskatoon during 2004 with further selection based on agronomic and grain quality evaluation with further selections advanced to 2005 and 2006 trials as indicated below. Best putative slow DMD hulled and hulless two-row selections with high SKCS grain hardness derived from WC795 and WC1064 have reached the Prelim MicroPlot Yield testing stage at Saskatoon in 2007, while the last of the high SKCS grain hardness selections derived from Valier crosses have reached either the replicated Prelim YT or multi-site replicated Standard YT stage of the program.

Unfortunately, based on the results of this overall project for Valier, while it is possible, we do not expect the Valier derivatives to provide germplasm consistently demonstrating the slow

DMD trait. We do believe that the WC795 and WC1064 derivatives may provide such germplasm, however, the proof of that will require in-vivo DMD rate evaluation using live animals which is not within the scope of this project in terms of time frame or cost.

Germplasm Development – Project Objective #2 – "To develop locally adapted barley germplasm with the slow DMD trait."

Due to the delays in the project caused by weather related issues in 2002 and again in 2004 the project was extended for the 2006 growing season. With that extension to include a fifth (2006) growing season the following sections report activity under the project as conducted during 2002 through 2006.

The 2005 growing season was characterized as average although harvest was again hampered and delayed by wet weather. Yields were above average, and while grain quality was somewhat compromised, grain quality was generally acceptable for post-harvest grain quality analysis critical to this project. The 2006 growing season was best characterized as benign with average yields of good quality grain as a result of excellent weather during the harvest season.

Valier special increase at KCRF 2002 & 2003 –

2002

2 acres grown to supply slow DMD grain for possible proof of concept animal feeding studies and to be available as seed in 2003 larger scale increases for future studies. Target yield = 4 tonnes, actual yield = 1.4 tonnes, with questionable seed quality due to sprout damage and significant level of immature kernels. Not useable for feeding trials. Salvage seed value at best.

2003

0.5 acres grown with 873 kgs clean seed produced for possible animal feeding trials or as seed for larger future increases.

2004, 2005, 2006

None grown

Valier, WC 1064 and WC795 Bulk Increases at KCRF –

2002

0.02 acre increases grown to produce seed for 2002/03 testing and 2003 increase. Target yield = > 50 kg, harvested yield = < 20 kg, with questionable seed quality due to sprout damage and significant level of immature kernels. . Not useable for feeding trials. Salvage seed value at best.

2003

0.02 acre increases of Valier, WC795 and WC1064 grown and harvested. 0.02 acre increase of SB02562 (putative CDC slow DMD selection) also grown and harvested. Approximately 40 kgs of excellent quality seed of each harvested and in store.

2004

0.02 acre increases of Valier, WC795 and WC1064 grown and harvested. 0.02 acre increase of SB02560, 2001-517-01-061 & 2001-517-01-123 (putative CDC slow DMD selections) grown and harvested. Approximately 40 kg of good quality seed of each harvested and in store.

2005

0.02 acre increases of SB03131 (putative CDC Slow DMD seln), WC795 and WC1064 grown and harvested. Approximately 30 kg of good quality seed of each harvested and in store.

2006

None grown

Non-replicated Preliminary Yield trials

2002

30 Valier x CDC Helgason and 24 Valier X TR253 SSD RIL selections grown at KCRF. As previously reported these trials became salvage increases with no field data collected. To attempt seed stock recovery a single 2.5 m plot of each of the 54 RILs plus plots of the checks Valier, TR253 and CDC Helgason was grown in the 2002/03 NZ winter nursery. Each NZ plot produced seed which was used for 2003 trials. An attempt was made to evaluate salvaged 2002 seed for the slow DMD trait and selections were advanced to 2003 trials on the basis of this questionable (due to effects of sprouting) data. Results from 2003 trials (2003 SDMDBYT #1 – Combined analysis attached) suggest that, as expected, this was a futile attempt and that the 2002 samples were simply too compromised to be of any value.

2003

2003 ALWALYT – 166 SSD derived RILs from the cross Valier x CDC Helgason grown in an unreplicated MADD design yield trial at Saskatoon (limited irrigation). Data was collected for grain yield, days to heading, test weight, kernel weight and % plump. With checks averaging some 3500 kg/ha the RIL population mean yield ranged from 2300 – 4400 kg/ha. In early 2004 these samples were evaluated for SKCS hardness and GPSA.

2004

2004 CAMMBYT – 166 SSD derived RILs from the cross Valier x CDC Helgason grown in an unreplicated MADD design yield trial at the Kernen Crop Research Farm. Data was collected for grain yield and days to head. Of note the mean yield for 13 plots of the highest yielding check CDC Helgason was 5,978 kg/ha and a number of the selections from the Valier x CDC Helgason population yielded at or above that level. Test weight, kernel weight and % plump measures collected and these samples, along with those collected from the same experiment grown in 2003, were evaluated for the slow DMD trait using the SKCS, GPSA and other methodologies developed elsewhere in the project.

2004 Micro-plot PFBYTs – 75 putative slow DMD selections from Valier x TR253 and Valier x CDC Helgason populations grown at Saskatoon. Post-harvest data collected with 12 selections advanced to further testing in 2005 PBYTs.

2005

2005 PBMP # 17 – 5 lines from Valier x TR357 and 23 from Valier x TR361 evaluated with two, SB050893 and SB050906) advanced to 2006 Prelim BYT #9.

2005 MiscBYT # 10 – 19 entries from Valier x CDC McGwire, 10 from WC795 x CDC Helgason and 9 from WC1064 x CDC McGwire evaluated with three Valier x CDC McGwire selections (SB041468, SB041205 & SB041212) advanced to multi-site 2006 SBYT# 2 or 3.

2005 MiscBYT # 13 – 7 entries from Valier x TR253. None advanced to 2006 testing.

2006

Prelim MicroPlot BYT #'s 5, 6 & 22 – 378 entries from 13 crosses (putative slow DMD parents WC795, WC1064, Valier and SB02562) evaluated at Saskatoon. 40 selections advanced to 2007 Prelim BYT#'s 14, 18 or 19.

MiscBYT # 5 – 30 entries from 2 crosses (putative slow DMD parents WC795 and WC1064) evaluated at Saskatoon. 9 selections advanced to 2007 Prelim BYT 18 or 20.

Replicated Preliminary Yield trials

2003

2003 SDMDBYT 1 – 6 RIL selections from the cross Valier x Helgason and 7 from the cross Valier x TR253 plus three checks grown in replicated yield trials at two locations. These RILs had been selected to represent sets of normal and slow DMD selections based on GPSA conducted on the compromised samples from a similar trial grown in 2002. However, based on SKCS hardness Index (see attached data file SDMDBYT 1), all selections from the Valier x Helgason population appear to be slow DMD while the RILs from the Valier x TR253 cross are more variable. However, that variability does not correlate with GPSA estimates from our 2002 trials with these selections. Despite that, based on SKCS hardness index, several selections appear to be slow DMD type and advanced to 2004 multi-site yield trials.

2004

2004 SDMDBYT#2 – 16 putative slow DMD selections plus four checks (Newdale, CDC Helgason, TR253 and Valier) grown in a 3 rep trial at the Kernen Crop Research Farm. Data collected included grain yield, days to head, visual rating, test weight, kernel weight and % plump. Chemical quality data and information related to the slow DMD trait collected with two selections 01T517-01-020 and 01T517-01-036 advanced to 2005 Standard YTs.

2005

2005 PFBYT # 1, 2, 3 and 4 – 7 lines from Valier x CDC Helgason and 5 lines from Valier x TR253 evaluated with SB040061 and SB040068 from Valier x CDC Helgason and SB04730 and SB040093 from Valier x TR253 advanced to multi-site 2006 SBYT 1 or 2.

2006

Prelim BYT # 9 – 2 entries from the cross Valier x TR361 evaluated at 2 sites. Neither selection advanced to 2007 testing.

Advanced multi-site replicated Standard YTs

2003

2003 SFBYT #1 – a single speculative slow DMD selection from the Valier x CDC Helgason population designated SB02562 was evaluated at 6 locations across western Canada. SB02562 performed well ranking 4/36 for yield with very good physical grain quality and reasonable disease resistance. Unfortunately we were unable to verify slow DMD status thus the line was dropped from further evaluation.

2004

2004 SDMDYT#1 – 12 putative slow DMD selections plus four checks (Newdale, CDC Helgason, TR253 and Valier) grown in a 3 rep trial at the Kernen Crop Research Farm, Goodale Crop Research Farm and at Harris, SK. Data collected included grain yield, days to head, visual rating, test weight, kernel weight and % plump (see attached). Of note were lower % plump values for the test materials relative to the checks. Chemical quality data and information related to the slow DMD trait collected with selections SB02563 and 01T517-01-013 advanced to 2005 Std YT. Unfortunately we were unable to verify slow DMD status thus these lines were dropped from further evaluation.

2004 SFBYT#3 – ***6 slow DMD selections grown as part of a 36 entry 3 rep trial at 6 locations KCRF, Stoon, Goodale, Brandon, Scott and Lashburn with selection SB03131 advanced to 2005 Eastern Prairie Barley YT.*** Unfortunately slow DMD evaluation tests indicated the selection was not slow DMD type thus the line was dropped from further evaluation.

2004 SFBYT#5 – 9 slow DMD project selections grown as part of a 36 entry 2 rep trial at KCRF and Stoon with selections SB03119 and SB03132 retested in 2005 Standard YT. Unfortunately we were unable to verify slow DMD status thus the line was dropped from further evaluation.

2004 SFBYT#7 – 3 slow DMD project selections grown as part of a 36 entry 1 rep trial at KCRF and Stoon with SB03123 retested in 2005 Standard YT. Unfortunately we were unable to verify slow DMD status thus the line was dropped from further evaluation.

2004 BarVYT – Valier, TR253 and CDC Helgason included as part of this 12 entry 3 rep trial grown at 7 sites across western Canada (Stoon, KCRF, Goodale, Wakaw, Watrous, Brandon and Lacombe). Samples from these trials used to evaluate the slow DMD trait of Valier versus TR253 and CDC Helgason (the other parents in the slow DMD project study populations) and to again evaluate the consistency of the slow DMD trait in Valier across different environments.

2005

SBYT 1, 3 and 4 – 4 lines from Valier x CDC Helgason and 3 lines from Valier x TR253 evaluated at six sites across western Canada. 01-T517-01-020 from Valier x CDC Helgason and SB031123 from Valier x TR253 re-evaluated in 2006 SBYT # 1 or 2. Not advanced for further testing in 2007.

2006

SBYT 1 & 2 – 9 hulled selections from 4 crosses with Valier as slow DMD parent evaluated at 6 sites across western Canada. None advanced to 2007 trials.

SBYT 3 – 3 hulless selections from the cross Valier x CDC McGwire evaluated at 6 sites across western Canada. None advanced to 2007 trials.

RIL hills plots

2002

Two replications of quadruple hill plots of 238 RILs from the cross Valier x TR253 and 168 RILs from the cross Valier x CDC Helgason were grown at the Preston Plots during 2002. Unfortunately rep 2 was lost to sprouting.

2003

Test 932-01 – Two reps of quadruple hill plots of 228 Valier x TR253 RILs planted, irrigated and harvested and stored for cleaning and physical and chemical quality data collection in 2004 (SKCS hardness, kernel weight and diameter data collected).

2004

Test 903 – 89 RILs from the cross WC1064 (slow DMD hulless 6 row) x CDC McGwire grown as 4x hills, 94 RILs from the cross WC795 (slow DMD hulless 6 row) x CDC Helgason and 124 RILs from the cross Valier (slow DMD hulled 2 row) x CDC McGwire grown at Saskatoon. Only two row segregates harvested with 43 hulless selections from the first cross; 15 hulless, 6 segregating and 7 hulled selections from the second cross and 48 hulless, 11 segregating and 31 hulled selections from the third cross retained. SKCS data collected indicated a wide range of grain hardness with values from < 45 to > 80 vs normal checks at 50 SKCS units. 61 selections advanced to 2005 MiscBYT # 9 and 10.

2004 Test 520-01 – 204 F5 SSD RIL hills from the cross Valier x TR357 with 5 and 15 selections respectively advanced to 2005 Prelim MicroPlot YTs or retested as 2006 F6 hill plots.

2004 Test 520-02 – 252 F5 SSD RIL hills from the cross Valier x TR361 with 23 and 22 selections respectively advanced to 2005 Prelim MicroPlot YTs or retested as 2006 F6 hill plots.

2005

2005 T-908 – 18 selections from WC1064 x CDC McGwire and 16 hulled and 17 hulless selections from WC795 x CDC Helgason evaluated with 32 selections advanced to 2006 MiscBYT # 5.

2006

None grown

Doubled haploid hill plots

2002

One replication of paired hill plots of 280 microspore derived doubled haploids from cross MC0052 (Valier x TR253) grown at the North Seed Farm. Unfortunately due to late emergence, early drought, heavy weed pressure, frost and sprouting these plots could only be salvaged for seed and it will be necessary to regrow these in multiple hill plots in 2003. This 2x haploid set is ideal for inheritance studies and searching for associated molecular markers.

2003

2003 Test 931-01 = two replications of paired hills of 247 doubled haploid lines from cross MC0052 (Valier x TR253) grown under irrigation at Saskatoon. Harvested and stored for cleaning and physical and chemical quality data collection in 2004 (SKCS hardness, kernel: weight and diameter). Based on parental analysis this large doubled haploid population will serve as the base material for much of the remainder of the methodology and inheritance sub-projects.

2004

Test 910 – two reps of 247 double haploid lines from the cross Valier x TR253 grown as 4x hills. Based on parental analysis this large doubled haploid population served as the base material for much of the remainder of the methodology and inheritance sub-project.

Test 904 – 10 Valier x TR253 doubled haploids selected for very high versus very low 2003 SKCS hardness scores grown as 4 replicate 4x hills at Saskatoon with checks Valier, TR253, CDC Bold and TR710.

2005

None grown

2006

None grown

Regular F5 & F6 hill plots

2003

2003 Test 506-02 – 163 F5 selections from the cross Valier x TR253 grown with approximately 50% field selected and harvested. Best selections advanced to 2004 Preliminary YTs or F6 hill trials.

2003 Test 605-01 & 02 – 82 F6 selections from the cross Valier x CDC Helgason and 73 from the cross Valier x TR253 grown with approximately 50% field selected and harvested. Best selections advanced to 2004 Preliminary YTs .

2004

None

2005

Test 507 – 1448 selections evaluated from 7 different crosses with Valier, WC795 and WC1064 as slow DMD parents. 499 field selected and harvested with 176 selections advanced to 2006 Prelim Barley MicroPlot YTs.

Test 512-01 – 216 lines from SB02562 x CDC Coalition with 74 field selected and 19 advanced to 2006 Prelim MicroPlot YT # 23.

2006

Test 524- 01 & 02 – 156 6R lines from the cross WC1064 x SH01042 and 160 lines from the cross WC1064 x AC Rosser evaluated. 19 and 10 selections respectively advanced to 2007 Prelim MicroPlot YTs.

Early generation slow DMD development *(putative slow DMD parent bolded)* :

New slow DMD crosses 2005:

06 F2 Test #	05 Cross #	Pedigree	Comments	2006 Comment
2070	72	**SH041062** x CDC McGwire	V hard hulless WC1064 derivative x hulless perf	Discard
2071	73	***SH041165** x TR05384	Very hard hulless WC795 derivative x hulled, yld, stg, gr?, net, spot	+ h only
2072	74	**SH041228** x CDC McGwire	Hard hulless Valier derivative x hulless perf	
2073	75	**SB041467** x TR05388	Hard hulled Valier derivative x v stg, sht, G gr, net, spot?, L smut	
2074	76	**SB041501** x TR05383	Hard hulled Valier derivative x yld, v stg, gr?, FHB, net	Discard
2075	77	**SB041436** x AC Rosser	Very very hard 6R hulled x 6R hulled perf	
2076	78	**SB041436** x TR05385	Very very hard 6R hulled x 2R, v stg, VG gr, FHB OK, net, spot	
2077	79	***SH041141** x CDC McGwire	Very very hard 6R hulless x 2R hulless, perf	
2120	80	**SH041141** x SH02028	Very very hard 6R hulless x 6R hulless perf	

- *F1's grown in 2005/06 NZ winter nursery or 2006 Stoon winter GH*
- *F2 bulks grown at Saskatoon 2006*
- *F3 bulks being grown in 06/07 NZ winter nursery*
- ** 06/07 F3/F4 SSD at Saskatoon*

New slow DMD crosses 2004:

06 F4 #	05 F2 Test #	04 Cross #	Pedigree	Comments	Comments
4006	227	28	*CDC Bold x **McLeod**	Very Low SKCS X Very High SKCS	
----	228	29	**SB02560** x TR04377	> SKCS slow DMD x FHB, stg, tall	Discard F2
4007	229	30	**SB02563** x CDC Mindon	> SKCS slow DMD x DON, VG gr, stg, L, smut, dis OK	Discard F4

- *F1's grown in 2004/05 NZ winter nursery or 2005 Stoon winter GH*
- *F2 bulks grown at Saskatoon 2005*
- *F3 bulks grown in 05/06 NZ winter nursery*
- *F4 space plant nursery Saskatoon 2006*
- *F5 hills 2007 Saskatoon*
- ** 05/06 F3/F4 SSD at Saskatoon with F5 RILs in 2006 T-535-01 with 256 RILs evaluated and 45 selections advanced to 2007 Prelim MicroPlot YTs.*

F2s grown 2004 (Crosses made in 2003):

06 F5	05 F4#	04 F2#	03 Cross #	Pedigree	Comments	Comments
524-01	444	228	39	WC1064 x SH01042	Sl DMD 6R X 6R hulless performance	
524-02	446	229	40	WC1064 x AC Rosser	Sl DMD X 6R performance	
-----	421	230	41	*SB02562 X CDC Coalition	Putative slow DMD 2R x yld	Discard F4
-----	448	231	42	SB02562 X WC1064	Putative slow DMD 2R x 6R slow DMD	Discard F4
-----	422	232	43	SB02562 X BM9534-115	Putative slow DMD 2R x performance	Discard F4

- *F1's grown in 2003/04 NZ winter nursery or 2004 Stoon winter GH*
- *F2 bulks grown at Saskatoon 2004*
- *F3 bulks in 2003/2004 New Zealand winter nursery*
- *F4 space plant nursery Saskatoon 2005*
- ** 2004/2005 U of S F3 F4 SSD with 150+ F5 RILs to 2005 T512-01 hill plots at Saskatoon – 216 entries evaluated, 19 selections advanced to 2006 PBMPYT 23 & 4 selections advanced to 2007 Prelim BYT #14.*
- *F5 hill plots at Saskatoon 2006 – 316 entries evaluated with 29 advanced to 2007 Prelim MicroPlot YTs.*

F4's grown 2004 (Crosses made in 2002)

05 F5 #	04 F4	03 F2	Pedigree	Comments
507-01	462	243	**CDC McGwire x WC795**	Sl DMD 6R X McGwire
507-02	463	244	**Valier**/McG F1 x McG (hulled)	Sl DMD McG BC
507-03	464	244	**Valier**/McG F1 x McG (hulless)	Sl DMD McG BC
507-04	465	245	**WC1064**/McG F1 x McG	Sl DMD 6x2 +h/-h X McG BC
507-05	466	246	***Valier** x TR357	Sl DMD X Yld, v stg, TKP
507-06	467	247	**WC1064**/Bold F1 x CDC Trey (hulled)	Sl DMD 6x2 X Yld, TK, G net, SFNB
507-07	468	247	**WC1064**/Bold F1 x CDC Trey (hulless)	Sl DMD 6x2 X Yld, TK, G net, SFNB
507-08	469	263	**WC795**/Helgason F1 x TR01362 (hulled)	Sl DMD 6x2 X Yld, smut, L, sht, stg?, G net
507-09	470	263	**WC795**/Helgason F1 x TR01362 (hulless)	Sl DMD 6x2 X Yld, smut, L, sht, stg?, G net
507-01	471	264	***Valier x TR361**	Sl DMD x Performance and FHB

- *F3's grown in 2003/04 NZ winter nursery*
- *F4 space planted popns at Saskatoon 2004*
- *Single head selections to 2005 T507 F5 hills at Saskatoon* – 1448 selections evaluated, 499 field selected and harvested with 176 selections advanced to 2006 Prelim Barley MicroPlot YTs and 35 advanced to 2007 Prelim BYTs.
- ** 2003/2004 SSD at Saskatoon – 204 and 252 RILs to 2004 F5 hills respectively as noted in RIL Hill Plots 2004 section above.*

F5's grown 2004 (Crosses made prior to project):

05 F6 #	04 F5 #	03 F3 #	Pedigree	Purpose
----	556	306	MC9813-08 x **Valier** (hulled)	Low phytate hulless x slow DMD
----	557	306	MC9813-08 x **Valier** (hulless)	Low phytate hulless x slow DMD
611-02	558	307	MC9813-20 x **Valier**	Low phytate hulled x slow DMD
611-03	559	308	**WC1064** x CDC McGwire	Slow DMD 6R hulless x Hulless 2R
611-04	560	309	**WC1064** x CDC Bold (hulled)	Slow DMD 6R hulless x Hulled 2R
----	561	309	**WC1064** x CDC Bold	Slow DMD 6R hulless x Hulled 2R
611-05	562	310	**WC 795** x CDC Helgason (hulled)	Slow DMD 6R hulless x Hulled 2R
611-06	563	310	**WC 795** x CDC Helgason (hulless)	Slow DMD 6R hulless x Hulled 2R
611-07	564	311	**WC1064** x SH99119	Slow DMD 6R hulless x Hulless 6R
611-08	565	312	**Valier** x CDC McGwire (hulless)	Slow DMD 2R x Hulless 2R
----	566	312	**Valier** x CDC McGwire (hulled)	Slow DMD 2R x Hulless 2R

F4 bulks grown in 2003/04 NZ winter nursery
2004 F5 space planted popns at Saskatoon with F5 single head selections
2005 F6 hills at Saskatoon – 1104 lines evaluated,133 selections advanced to 2006 Prelim MicroPlot YT and 15 advanced to 2007 Prelim YTs.

F4's SSD hills grown 2003: to F6 multi hills in 2004.

03 F4 #	# Hills	Pedigree	Purpose
499-03	164	**WC1064** x CDC McGwire	Slow DMD 6R x hulless 2R
499-02	164	**WC795** x CDC Helgason	Slow DMD 6R x 2R performance
499-03	206	**Valier** x CDC McGwire	Sow DMD 2R x hulless2R

All grown as single F5 SSD hills in 2003/04 NZ winter nursery and grown as multiple F6 hills at Saskatoon in 2004 as described in RIL hill plots 2004 section of this report.

F5 hills grown 2003 to 2004 PFBYTs.

03 F5 #	Pedigree	Purpose	# of hills
506-01	**Valier** x unknown 6R	Slow DMD x 6R	128
506-02	**Valier** x TR253	Sow DMD x hulled 2R	163

- 19 & 35 selections advanced to 2004 PrelimYTs as described above

F6 hills grown in 2003 to 2004 PFBYTs.

03 Test #	Pedigree	# of Hills	Comments
605-01	**Valier** x CDC Helgason	82	1 head per hill salvage
605-02	**Valier** x TR253	73	1 head per hill salvage

- 19 & 21 selected lines to 2004 StdYTs as described above.

Special slow DMD replicated 4x hill trials 2003 and 2004.

03 Test #	Pedigree	# of Hills
932-01	**Valier** x TR253	228 4x hills replicated 2x

- *re-grown as replicated 4x hill plots in 2004 as described above.*

Special doubled haploid slow DMD hill trials 2003 and 2004.

03 Test #	Pedigree	# of Hills
908-03	**Valier** x Unknown 6R	22
931-01	**Valier** x TR253 (MC0052)	247 2x hills x 2 reps

- *selections 2003 908-03-6, 13, and 18 tested in 2004 2x haploid PFBYT or microplot YT*
- *selections 2003-908-03-5 and 19 evaluated in 2004 2xhapPFBYT#32*
- *247 2x haploids from 2003-931-02 re-tested in 2 rep multi hill plots at Saskatoon in 2004.*

Equipment - none purchased

Project Developed Materials - none

Project publications –

Oral:

G. Camm. 2006. Grain Processing Differences between Barley Varieties for Cattle. Plant Sciences Int'l Grad Student Symposium. Saskatoon. March.

Posters:

G. Camm and B. Rossnagel. 2005. Grain Processing Differences between Barley Varieties for Cattle. Saskatchewan Soils and Crops Workshop. Saskatoon. March.

G. Camm and B. Rossnagel. 2005. Milling Energy and Grain Hardness in Barley. North American Barley Researchers Workshop. Red Deer. July.

G. Camm and B. Rossnagel. 2007. Heritability of SKCS Grain Hardness in Barley. 5th Canadian Barley Symposium the Science and Joy of Canadian Barley and Beer. Winnipeg. June.

G. Camm and B. Rossnagel. 2007. Grain Hardness in Barley. Saskatchewan Soils and Crops Workshop. Saskatoon. March.

Thesis:

G. Camm. 20072008. Grain Hardness and Slow Dry Matter Disappearance Rate in Barley. MSc thesis to be submitted to the College of Graduate Studies and Research. University of Saskatchewan.

Project photos – none

Acknowledgement - the ADF contribution is recognized each time reports are developed and each time the project is noted during various extension events and media opportunities.

Attachments

None